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5123

An Experimental Study of the Factors Concerned in Mammary Growth and in Milk Secretion.

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Greuter[1] has recently reported that the injection of anterior pituitary extracts into lactating cows and goats increases the flow of milk. Corner[2] in a recent demonstration has shown that it is possible to initiate the milk flow in oöphorectomized adult virgin rabbits by injecting an anterior lobe extract.

At the time of these reports the present authors were engaged in a study of the factors concerned in mammary growth and milk secretion. The animals employed were normal and castrated immature male and spayed immature female guinea pigs. The animals were given a preliminary treatment consisting of daily subcutaneous injections of a lipid extract of sows' corpora lutea. This treatment produced a very marked hypertrophy of the glands and nipples as was demonstrated both macroscopically and by histological study. In no instance (12 animals) was milk secreted although in some cases injection was continued for a month. If, however, the growth treatment was followed by injection of anterior pituitary substance, milk was secreted within 3 days (7 animals). Since either fresh gland (rat) or an extract of sheeps' glands containing the maturity hormone* proved efficacious in this respect it seems probable that the maturity hormone of the pituitary is the one involved in this

[1] Greuter, F., Paper read before the Second International Congress for Sex Research, London, 1930.

[2] Corner, G. W., *Anat. Rec.*, 1930, **45**.

* The anterior pituitary extract employed in this work was supplied through the courtesy of Dr. Oliver Kamm of Parke, Davis & Co.

reaction. It is possible, of course, that an at present unknown principle of the anterior lobe rather than the maturity hormone is responsible, but the nature of the material used in this work is indicative of the latter.

A series of controls (8 immature males and females) was injected with the pituitary extract only. In no instance was milk produced nor were the mammary glands hypertrophied during the period of injection (5 to 6 days).

Preliminary work employing oestrin in the growth treatment has been carried out on immature males. Although considerable hypertrophy of the mammary glands was obtained in the 4 animals subjected to this treatment, subsequent injection of the pituitary extract has so far proved unsuccessful in initiating milk secretion. Further work along this line is in progress.

That the factor contained in the lutein extract is not oestrin has been shown by adequate controls. Oestrin may be present, but not in sufficient quantities to respond to physiological tests.

The authors have been able to confirm Corner's work,[2] using adult virgin guinea pigs which were gonadectomized during oestrus. A copious flow of milk was produced in 3 animals on the second or third day after the treatment was begun. The only agent employed was the anterior pituitary extract referred to above.

The results obtained to date indicate that the mammary glands must first be developed to a proper state by some ovarian factor before the pituitary principle can initiate secretion. On the other hand, ovarian factors alone are not sufficient. These indications are suggestive of the rôle that may be played by the ovarian and pituitary hormones in normal milk production.

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Further Note on a Substance in Liver Active in Pernicious Anemia.

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A method has already been described[1] for the separation from liver of an acidic substance clinically potent in pernicious anemia. The substance on hydrolysis was found to yield β-hydroxyglutamic acid and evidence was also obtained of the presence in the hydrolytic

[1] *J. Biol. Chem.*, 1930, **88**, 427.

products of a neutral laevorotatory compound precipitable at least in part by phototungstic acid. This latter substance has been identified as *l*-γ-hydroxyproline. It was characterized by its specific laevorotation, absence of amino nitrogen, phenylisocyanate derivative M. P. 170° and copper salt. The free acid appears identical with the product obtained by the hydrolysis of proteins. The mode of linkage of the hydroxyproline with hydroxyglutamic acid is under investigation.

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Growth of Rickettsia of Typhus Fever (Mexican Type) in the Presence of Living Tissue.

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In spite of several attempts to cultivate the virus of typhus fever by the method of tissue culture, it seems that such cultures could not be carried through repeated successive generations. The methods employed have been practically the same; *viz.*, the cultivation of tissues (generally brain and spleen) from typhus infected guinea pigs in homologous plasma. Thus Kuczynski[1] found the virus of typhus fever virulent for 4-19 days, Krontowski and Hach[2] for 8 days, Wolbach and Schlesinger[3] for 20 days (28 days by transferring the same piece of tissue into fresh medium), and Rix[4] for 6 days. Recently Zinsser and Batchelder,[5] using tunica tissue from testicles of guinea pigs infected with Mexican typhus, prepared cultures which were virulent for one week. They were able to demonstrate rickettsias in great numbers in smears from such cultures.

The strain of typhus used in our studies, was isolated from a case in the southeastern United States by the Hygienic Laboratory in Washington, D. C. This strain is in all respects quite similar to the Mexican strain of Mooser. While not all of our attempts to

1 Kuczynski, Max H., *Berl. klin. Wochenschr.*, 1921, **2**, 1489.

2 Krontowski, A. A., and Hach, I. W., *Münch. med. Wochenschr.*, 1923, 144; *Klin. Wochenschr.*, 1924, **2**, 1625; *Arch. f. exp. Zellforsch.*, 1926-27, **3**, 297; *Z. f. Immunitätsforsch.*, 1927-28, **54**, 237.

3 Wolbach, Burt and Schlesinger, Monroe, J., *J. Med. Res.*, 1923-24, **39**, 231.

4 Rix, Erich, *Z. f. Hyg. and Infek.*, 1928, **108**, 103.

5 Zinsser, Hans, and Batchelder, Albert P., *J. Exp. Med.*, 1930, **51**, 847.

establish strains by the methods to be described were successful, the following results were obtained in a number of experiments.

Employing the technic of Rivers, Haagen and Muckenfuss[6] for the cultivation of the viruses of vaccinia and herpes in tissue cultures of rabbit cornea in coagulated plasma, it was possible to carry the virus of typhus fever through at least 7 generations covering a period of 10 weeks. Pieces of normal guinea pig tunica (about 2 mm. square), soaked for a few minutes in a saline suspension of tunica scrapings from guinea pigs infected with typhus, were imbedded in wide tubes in a medium of guinea pig plasma coagulated by means of saline extracts of normal guinea pig spleen.

The successful cultivation of vaccinia virus by Maitland and Maitland[7] and by Rivers, Haagen and Muckenfuss[8] in a liquid medium composed of Tyrode solution, serum and minced tissue, suggested the possibility of using this same medium for the cultivation of typhus virus. Thus cultures were prepared as follows: minced normal guinea pig tunica, inoculated with typhus tunica scrapings as for the piece cultures above, was suspended in the serum-Tyrode medium of Maitland and distributed in about 3 cc. amounts in 25 cc. Erlenmeyer flasks (as suggested by Rivers). This medium was found to be as satisfactory for the growth of typhus virus as the coagulated plasma medium used above for the single piece cultures. By this method the virus was carried through 6 generations covering a period of 8 weeks.

Although our experiments were interrupted during the summer, it would seem that the cultures could be continued in either way indefinitely.

Transfers for both types of cultures were made at 8-12 day intervals by removing the tissue from the medium and inoculating fresh normal tunica with a small amount of the cloudy liquid obtained by scraping the issue of the previous culture.

Even the last generations of the cultures showed large numbers of rickettsias in Giemsa stained smears, the microscopical picture being like that published by Zinsser and Batchelder.[5]

Entirely characteristic infections with good scrotal swelling could be produced in guinea pigs upon intraperitoneal injection of single pieces of tissue (about 1 mm. square) from both types of cultures whenever tested; *e. g.*, from the fifth generation Maitland cultures.

[6] Rivers, T. M., Haagen, E., and Muckenfuss, R. S., *J. Exp. Med.*, 1929, **50**, 665. *Cf.* Carrel, Alexis and Rivers, T. M., *Compt. rend. Soc. Biol.*, 1927, **96**, 848.

[7] Maitland, H. B., and Maitland, M. C., *Lancet*, 1928, **2**, 596.

[8] Rivers, Haagen and Muckenfuss, *J. Exp. Med.*, 1929, **50**, 181.

The organisms described by Mooser were found in large numbers in smears made from the tunica exudate of these animals.

The question of whether live tissue is necessary for the growth of typhus organisms, as it is supposed to be in the case of filterable viruses (*cf.* Rivers), is being studied by using tissues killed in various ways; *e. g.*, by heating, by repeated freezing and thawing, by anaerobiosis, etc.

5126

The Excretion of Xylose by Glomerular and Aglomerular Kidneys.

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Marshall[1] in a comparative study of the function of the glomerular and aglomerular kidney found that glycosuria is easily produced in fish with glomerular kidneys, but only a trace of glucose ever appears in the urine from an aglomerular kidney, even when the blood sugar is high and phlorhizin is given. This observation with those of Corley[2] and Fishberg[3] on the rate of disappearance of xylose from the blood suggested that a foreign sugar such as xylose might serve as a basis for measuring the extent of filtration and reabsorption by the kidney. First it was necessary to establish that xylose is not excreted by the aglomerular kidney except in the very faintest traces. That is the object of this paper.

Four species of fish were selected. The cod fish *(Gadus callarias)* and the puffer *(Spheroides maculatus)*, fish with glomerular kidneys; the toadfish *(Opsanus tau)* and the goosefish *(Lophius piscatorus)*, fish with aglomerular kidneys, were studied. The experiments on the goosefish were performed by Dr. E. K. Marshall, Jr., at Salisbury Cove, Maine. The other fish were obtained at the New York Aquarium. The urinary papilla was tied off and xylose in aqueous solution was injected into the posterior dorsal muscles in all experiments except those on the goosefish in which the xylose was injected intravenously.[4] At the conclusion of the experiment, 1 to

[1] Marshall, E. K., Jr., *Am. J. Phys.*, 1930, **84**, 1.

[2] Corley, R. C., *J. Biol. Chem.*, 1926, **70**, 521.

[3] Fishberg, E. H., *J. Biol. Chem.*, 1930, **86**, 665.

[4] Marshall, E. K., Jr., and Grafflin, A. L., *Bull. Johns Hopkins Hosp.*, 1928, **43**, 203.

23 hrs. later, the fish were sacrificed and the urine in the bladder obtained by dissection. A filtrate, using 1 cc. of urine (1 cc. of a 1-50 dilution in the puffer series), was prepared by Somogyi's method.[5] Total sugar was determined on aliquot portions of this filtrate by the Hagedorn-Jensen Micro method. The rest of the filtrate was treated with washed baker's yeast as recommended by Van Slyke and Hawkins[6] and the non-fermentable reducing substances determined on aliquot portions.

The injections of xylose in the glomerular cod fish increased the non-fermentable reducing substances in the urine three fold (see table). In the glomerular puffer this increase was thirty-five fold.

TABLE I.

Species	Average Weight	Amt. of Xylose Injected	Non-fermentable reducing substances in the urine in mg. %	
			Before Injection of Xylose	After Injection of Xylose
Glomerular		gm.		
Cod fish	2 K.	1.0	34—53 Av. (3 fish) 41	84—185 Av. (4 fish) 123
Puffer	200 gm.	0.5	17—25 Av. (6 fish) 20	294—1176 Av. (6 fish) 705
Aglomerular				
Toad fish	300 "	0.5	22—77 Av. (3 fish) 42	39—82 Av. (6 fish) 56
Goosefish	12.7 K.	10.0	32	1st hr.....40 2-18 hr.....41

In the aglomerular toadfish and goosefish the increase in non-fermentable reducing substances was hardly beyond the range of normal variation.

Conclusions. In fish with aglomerular kidneys only the faintest traces of xylose appear in the urine following intramuscular or intravenous injection of xylose. In fish with glomerular kidneys injected xylose is readily recovered in the urine.

[5] Somogyi, M., *J. Biol. Chem.*, 1930, **86**, 655.

[6] Van Slyke, D. D., and Hawkins, J. A., *J. Biol. Chem.*, 1929, **83**, 51.

5127

The Action of Chlorine Compounds on B. Tuberculosis.

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The influence of compounds containing active chlorine on *B. tuberculosis* (strain H-37 obtained through the courtesy of Dr. S. A. Petroff) suspended in tap water has been determined by guinea pig inoculation. This investigation comprises 3 phases: first, a determination of compounds containing active chlorine germicidal to *B. tuberculosis;* secondly, the effective concentration of such compounds; and thirdly, the time of exposure required for compounds containing active chlorine in effective concentration to kill *B. tuberculosis.*

The compounds containing active chlorine were tested in amounts ranging from 10 to 100 parts per million, generally at increments of 10 parts per million. The time of exposure usually ranged from 15 seconds, 30 seconds, 1 minute, 3 minutes and 5 minutes.

The most practical disinfectant in low concentration appears to be chlorine gas in water in a concentration of 30 to 50 parts per million when applied for 5 minutes. Within the limits of investigation, the results are applicable to disinfection of eating and drinking utensils in restaurants, soda fountains, drinking places, etc., where contamination with *B. tuberculosis* is encountered.

Before reaching a final conclusion it will be necessary to investigate the influence compounds containing active chlorine in *B. tuberculosis* present in colloidal suspensions as well as in tap water.

5128

Serological Studies in Experimental Poliomyelitis.*

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The experiments briefly reported here are based on the observation that serum from poliomyelitic monkeys prevents the precipi-

* Under a grant from the International Committee for the study of infantile paralysis, whose work is being financed by J. Milbank.

† The author is indebted to Miss Florence Oram for technical assistance in this work.

tating effect of a gold chloride solution on tissue emulsions, particularly brain and cord extracts, while normal monkey serum apparently lacks this property. The phenomenon depends on observing certain quantitative relations, thus limiting the zone of comparable reactions to a definite experimental range. The stabilizing property of the immune serum, which is acquired comparatively early in the disease and tends to diminish somewhat in convalescence, is evidently an expression of the acute infectious process. Since the antigen in the test need not be specific, we have no way of telling whether the reaction is limited to the poliomyelitic infection exclusively. Control tests with sera obtained from monkeys inoculated with normal brain and cord and from monkeys subjected to extensive intradermal inoculation with vaccinia virus, so far have been negative.

The technique adopted after numerous variations tentatively as final, is briefly as follows: Three test tubes are filled each with 1.5 cc. of the supernatant of a centrifuged 5% monkey brain-cord emulsion. 0.2 cc., 0.15 cc. and 0.1 cc. of serum are added to the respective tubes, the volume in each tube then being equalized to a total volume of 2 cc. by the addition of corresponding amounts of 0.4% salt solution. Saline of the same strength is used in preparing the tissue emulsion. After 1 hour's contact, 0.075 cc. of a 1% gold chloride solution is added to each tube, care being taken to ensure an even mixture of all ingredients. After standing at room temperature for one hour the results are recorded. The test is profitably read again next morning.

In examining a total of 66 poliomyelitis sera from all stages of the disease and a total of 23 normal sera with this method, we have found the test readily allows a reliable distinction between normal monkey sera and poliomyelitis immune sera as evidenced by the following table.

TABLE I.

Results of stabilization test with monkey sera.

Total number of sera examined	Negative	Intermediate	Positive
66 poliomyelitis sera	0	12 (18.2%)	54 (81.8%)
23 normal sera	18 (78.2%)	5 (21.8%)	0

Code: Negative = complete or almost complete precipitation in the first two tubes.
Positive = complete or almost complete stabilization in the first two tubes.
Intermediate = partial precipitation in the first two tubes.

We have had occasion to follow the serum reaction in the same animal before and after infection in 10 instances. In all cases there

was the characteristic change of the serum reaction, occurring occasionally as early as the second day of the disease and increasing in strength as the intensity of the symptoms progressed. The question naturally presents itself as to the mechanism of the phenomenon. Is it an antigen-antibody reaction? Or are we merely dealing with an incidental unspecific increase in the protective colloidal properties of the serum, strangely characteristic for the poliomyelitic infection? We can offer no illuminating information as yet on that point. From the data on hand, we believe the serum change to be independent of a simple increase in serum globulins. In one case, we have succeeded in removing the stabilizing property of the immune serum after contact with virus cord, the serum after absorption reacting like a normal serum. It appears that the lipoidal substances in the nervous substance play the essential part in the antigen since fair results may also be obtained by substituting an alcoholic brain extract, diluted in suitable proportions for the watery emulsion. The stabilizing property of the immune serum was not destroyed by heating to 65° C. for ½ hour.

Human sera examined with the test show in the vast majority of cases the reaction of the poliomyelitis immune monkey serum, only 4% lacking stabilizing properties completely. We have found no relation between the Wassermann reaction and the result of the stabilization test in these sera. A comparison between sera from adults and sera from younger children of the susceptible age groups, although indicating a slightly higher percentage of non-stabilizing sera in the latter, has so far not brought out any really fundamental difference. We have tried to determine by crucial test whether the stabilizing property of the serum may be taken as an index for susceptibility in the human, as is obviously the case in the monkey, by examining a stabilizing and a non-stabilizing human serum for content of viruscidal substances. (0.5 cc. of serum was mixed with 0.5 cc. of virus emulsion and after incubation for 1 hour at 37° C, was left in the icebox overnight, each of the mixtures then being injected intracerebrally into a monkey.) While one experiment carried out so far seems to support such an hypothesis, further work must be done before we are in a position to draw more definite conclusions.

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A Comparison of the Efficacy of Different Methods of Active Immunization in Experimental Poliomyelitis.*

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The difficulties encountered in obtaining protection against poliomyelitic infection in the monkey with any degree of regularity by the various recognized methods of active immunization are sufficiently known to any worker in this field.[1, 2, 3, 4] In the following paper we have compared the efficacy of different routes of administration of the virus in the production of immunity against intracerebral infection.

A total of 16 monkeys were divided in small groups of from 4 to 2 animals, a different method of immunization being utilized in each instance. Thus, 4 monkeys were immunized intradermally, 3 intrapleurally,[5] 2 intraperitoneally, and one subcutaneously, while 2 monkeys received the virus intrarectally after thorough cleansing of the colon.[6] From 2 to 4 injections were given in the various groups, at intervals of 2 to 3 weeks. Live virus was used throughout this work, the size of the dose varying from 1 cc. to 3 cc. of a 20% cord suspension. Taking into account the density of the suspension, considerable amounts of virus were injected in some cases. In a last group of 4 monkeys, collodion sacs containing 3 cc. of virus suspension were inserted into the peritoneal cavity of the animals and left there indefinitely. The sacs, prepared according to the method described by Eggerth,[7] were sterilized before use in the autoclave (10 min. at 10 lb. pressure). They were roughly tested for permeability with a scale of different dyes of increasing particle size. The sacs which we used retained prussian blue, but allowed the passage of congo red in small quantities and were completely permeable for methylene blue. All animals were tested for immunity about one month af-

* Under a grant from the International Committee for the study of infantile paralysis, whose work is being financed by Jeremiah Milbank.

1 Aycock, W. L., and Kagan, J. R., *J. Immunol.*, 1927, **14**, 85.

2 Stewart, F. W., and Rhoads, C. P., *J. Exp. Med.*, 1929, **49**, 959.

3 Rhoads, C. P., *J. Exp. Med.*, 1930, **51**, 1.

4 Thompson, R., *J. Exp. Med.*, 1930, **51**, 777.

5 Gay, F. P., and Holden, M., *J. Inf. Dis.*, 1929, **45**, 415.

6 Hazen, E. L., *J. Immunol.*, 1927, **13**, 171.

7 Eggerth, A. H., *J. Biol. Chem.*, 1921, **48**, 203.

ter the last immunizing injection by intracerebral inoculation (1 cc. of a 5% virus cord emulsion), the virulence of the virus being controlled in each instance by simultaneous infection of one normal monkey. Details of experimental procedures and the results are conveniently tabulated in Table I.

TABLE I.

Comparison of different methods of active immunization.

Group	Monkey No.	Route of Immunization	No. of Injections	Amounts of virus injected cc.(20%)	Results during Immunization	Immunity as determined by Intracerebral Infection
I	49	Intradermal	2	7	Polio. 5 days	—
	76	"	2	7.5	—	Polio. 10 days
	77	"	3	8	—	" " "
	90	"	3	9.5	—	*No symptoms**
II	101	Intrapleural	3	3	—	*No symptoms*†
	102	"	3	1.5	—	*No symptoms**
	111	"	3	1.5	—	Polio. 9 days
III	170	Intraperitoneal	4	2	—	" " "
	192	"	4	2	—	" 7 "
IV	193	Subcutaneous	4	2	—	" 10 "
V	195	Intrarectal	4	4	—	" 7 "
	198	"	4	4	—	" 8 "
VI	86	Collodion sac	1	3	—	" 10 "
	88	" "	1	3	—	" 8 "
	89	" "	1	3	—	*No symptoms**
	122	" "	1	3	—	Polio. 7 days
	135	Control I, II	—	—	—	" 8 "
	182	" III, IV, V	—	—	—	" 7 "
	114	" VI	—	—	—	" 10 "

*These animals succumbed to poliomyelitis when reinfected one month after the first infection.

†This animal died from tuberculosis before reinfection.

It appears from Table I that one out of 4 monkeys immunized by the intradermal route was protected against the first infection, while one animal developed poliomyelitis during the period of immunization. Similarly, one monkey of 4 which carried virus-collodion sacs in the peritoneal cavity, withstood the first infection without showing any symptoms of the disease. The highest immunity index was obtained in a group of monkeys immunized with small doses of virus in the pleural cavity, 2 of 3 animals thus treated being fully protected against the first infection. Judging from the poor results obtained with intraperitoneal and subcutaneous immunization, it would seem that the success of intrapleural immunization was at-

tributable to the route of administration rather than to the size of the dose employed. As regards the possibilities of intrarectal immunization, with or without previous sensitization with bile, our results clearly indicate the inefficacy of such a procedure. The small number of experimental animals, in part due to intercurrent deaths, precludes the drawing of more definite conclusions at this time.

We wish to state in passing, that 6 monkeys, not enumerated above, which carried collodion sacs with virus in the peritoneal cavity, died with symptoms of progressive toxic cachexia in the course of 2 to 4 weeks. All of these monkeys were awkward in climbing, showing not only marked weakness of the legs and arms but lack of coordination of movements, and in 4 we observed a faint head tremor. The autopsy findings demonstrated the absence of lung infections (tuberculosis, pneumonia) and enteric infection (parasites). In some cases we found a slight congestion of the cord; histological examination however was essentially negative, as was transfer of the cord to a new monkey. The significance of these observations at present is obscure; they are especially perplexing inasmuch as our previous experience indicates that the virus in collodion sacs in the peritoneal cavity of the monkey is not viable beyond a period of 48 hours, contrasted with a survival for 10 days in the rabbit under similar experimental conditions. Work is under way to determine more accurately the actual length of survival and the occurrence of any possible changes in the nature of the virus in such sacs.

5130

A Critique of Einthoven's Law in Electrocardiography.*

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It is stated in authoritative books on electrocardiography that Einthoven's Law is mathematically exactly true. I wish to discuss this statement and to show that it is not correct. It is true that Einthoven's Law is approximately correct,[1] and that its practical use-

* Aided by the Emil and Fanny Wedeles Fund of the Michael Reese Hospital for the Study of Diseases of the Heart and Circulation.

[1] Pardee, H. E. B., Clinical Aspects of the Electrocardiogram, (Hoeber) 1928, 166.

fulness is unimpaired; the authoritative misstatement quoted above should nevertheless be refuted for the sake of scientific accuracy. I shall first define Einthoven's triangle and develop Einthoven's Law, and shall then show its inaccuracies.

It is considered that an electrocardiographic lead records the projection on a single line, called *the line of the lead,* of the cardiac potential vector; this vector is the sum of all the individual vectors of the heart muscle fibers. The 3 lines of the 3 ordinary leads of clinical electrocardiography are considered as lying all in one plane, so that they determine a triangle $A_1A_2A_3$ (Fig. 1); this is Eintho-

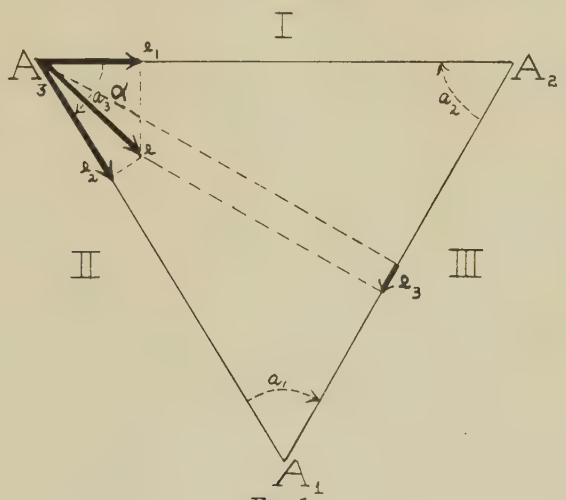

FIG. 1.

ven's triangle. In developing Einthoven's Law, it has been assumed that this triangle is equilateral; we gain something, however, by not making this assumption. Let the angles of the triangle be a_1, a_2, a_3. Let the projection of the cardiac potential on this plane be E; for convenience we represent E as directed from the point A_3, which may be done by shifting E parallel to itself, an operation which leaves unaltered its 2 properties, namely length and direction. Let e represent the length of E, and let α be the angle in a clockwise direction between A_3A_2 and E. It may be mentioned that whereas ordinarily E is spoken of as the electrical axis and α as the angle of this axis, this is not correct, since E is only the projection of the true electrical axis on this plane. The leads I, II, III record the projections e_1, e_2, e_3 of E on the lines A_3A_2, A_3A_1, A_2A_1. The vector E is constantly changing, and the corresponding changes of e_1, e_2, e_3 yield the electrocardiographic curves.

From Fig. 1 it follows by elementary trigonometry that

$$(1)\quad \begin{cases} e_1 = e\cos\alpha, \\ e_2 = e\cos(a_3 - \alpha), \\ e_3 = e\cos(180° - a_2 - \alpha) = e\cos(a_2 + \alpha). \end{cases}$$

From (1) it is readily proved that

$$(2)\quad e_2 \sin a_2 = e, \sin a_1 + e_3 \sin a_3;$$

this relation between e_1, e_2, e_3 is valid at every instant of the cardiac cycle.

If we assume, with Einthoven, that the triangle is equilateral, *i. e.*, that $a_1 = a_2 = a_3 = 60°$, then it follows at once from (2) that

$$(3)\quad e_2 = e_1 + e_3,$$

which is *Einthoven's Law.* This is well known, and has been demonstrated before, but the converse has apparently been overlooked, namely, that if (3) is true then the triangle is equilateral. The proof of the converse is as follows: If (3) is true then it follows from (1) that

$$(4)\quad \cos\alpha(\cos a_2 + \cos a_3 - 1) = \sin\alpha(\sin a_2 - \sin a_3);$$

since this holds throughout the cardiac cycle, it must be true for all α of the cycle, so that

$$\begin{cases} \cos a_2 + \cos a_3 - 1 = 0, \\ \sin a_2 - \sin a_3 = 0, \end{cases}$$

whence $a_2 = a_3 = 60°$, so that the triangle is equilateral. Consequently *Theorem: A necessary and sufficient condition for Einthoven's Law is that Einthoven's triangle be equilateral.*

We shall now discuss Einthoven's Law and the statements made concerning it. It has been stated that Einthoven's Law is necessarily true *a priori;*[2, 3] this statement is obviously based (erroneously) on the law of addition of forces, with which it has nothing to do; for e_1, e_2, e_3 are not independent forces but the projections of a single force. It is also stated that by observation Einthoven's Law is found to hold *exactly;*[4] this is simply based on insufficiently accurate measurements.

If Einthoven's Law held exactly, it would follow from our theorem that Einthoven's triangle is *exactly* equilateral. It would be an astounding biophysical fact if the arbitrary triangle obtained in every individual by using the arms and the left leg were exactly equilateral; indeed in this light it is clear *a priori* that Einthoven's Law can *not* hold exactly. There are further reasons for this view:

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[2] Fahr, G., *Arch. Int. Med.*, 1921, **27**, 126.

[3] Lewis, T., The Mechanism and Graphic Registration of the Heart Beat. (Shaw & Sons, London), 3rd Ed., 1924, 107.

[4] Wenckebach und Winterberg, *Die Unregelmässige Herztätigkeit.* (Englemann, Leipzig), 1927, 90.

1. An electrocardiographic lead does not record accurately the projection on a single line of the cardiac potential, because (a) much of the potential from the heart never reaches the electrodes but goes to distant parts of the body; (b) the electrical resistance of the tissues between the heart and the electrodes is not to be neglected and is a variable quantity. The error thus introduced is not necessarily the same or proportional in the 3 leads, so that it may alter the relations between e_1, e_2, e_3. 2. The line of a lead may not be in the same plane as the line joining the 2 electrodes of the lead. For example, the lead AB (Fig. 2) records the current whose path

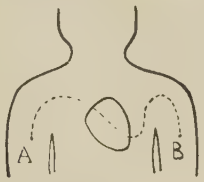

FIG. 2.

is represented by the dotted line, and this path may be largely in a direction not in the same plane as AB. Thus the 3 lines of leads I, II, III may not all be in one plane, so that there may actually not be any Einthoven triangle. 3. Even if there were an Einthoven triangle, it probably would be a changing one because the line of a lead may change slightly as the electrical axis moves; so that if the triangle were equilateral it would not remain so.

These points must make it clear that Einthoven's Law cannot hold exactly. It is enough that it holds approximately, and we should be satisfied with this and with its practical usefulness without claiming for it that which it can not possibly possess.

5131

Effects of Gonad Stimulating Extracts on Basal Gaseous Metabolism in Rats.

M. O. LEE AND JULES GAGNON.

From the Memorial Foundation for Neuro-Endocrine Research, Harvard Medical School, Boston.

Reiss and Winter,[1] using extracts containing the sex maturity stimulating principle from the urine of pregnant women, found no

[1] Reiss, M., and Winter, K. A., *Endokrinologie*, 1929, **3**, 174.

constant effects on the oxygen consumption, carbon dioxide production or respiratory quotient in rabbits. They used anesthetized animals, however, gave enormous doses, 200 to 4000 units of the material subcutaneously and followed the metabolism for only a few hours after each injection. We have used what are presumably more physiological doses, without anesthesia, and have followed the metabolic level during and after periods of continued daily administration.

Two sources of the sex maturity or gonad stimulating principle were used. One was an extract of sheep anterior lobe* and the other we prepared from the urine of women in the first half of pregnancy after the method of Biedl.[2] The potency of the extracts used was assayed by testing on infantile rats. Twelve rats were used, 9 females and 3 males. Twenty determinations made in the first 1 to 4 hours following the subcutaneous administration of the extracts in doses of 2 to 8 units showed no significant changes in the metabolic rates, although the tendency was towards an increase. No effects on the respiratory quotient were found.

These same rats were given doses of 1 to 6 rat units per day for periods of 8 to 24 days. Frequent metabolism determinations made during and after the periods of treatment showed no significant changes from the preliminary average level. The total average of the metabolic rates during the period of administration of the extracts was only 2% higher than the normal average rate. These results would indicate that in rats this material is without any general metabolic effect.

5132

Effect of Growth Promoting Extracts of the Anterior Pituitary on Basal Gaseous Metabolism in Rats.

M. O. LEE AND JULES GAGNON.

From the Memorial Foundation for Neuro-Endocrine Research, Harvard Medical School, Boston.

Lee, Teel and Gagnon[1] have previously reported that in giant rats produced by the daily administration of alkaline extracts of beef

* Prepared by Parke, Davis & Co.

[2] Biedl, A., *Endokrinologie*, 1928, **2**, 241.

[1] Lee, M. O., Teel, H. M., and Gagnon, J., PROC. SOC. EXP. BIOL. AND MED., 1929, **27**, 23.

anterior lobe pituitary, the basal gaseous metabolism was found to be somewhat reduced. We have made a further study of the effects of similar extracts in rats before there has been a great increase in weight and before the marked splanchnomegaly has had time to develop.

The immediate effects of growth stimulating extracts* were determined in 11 experiments on 3 male and 4 female rats. Doses of 1 or 2 cc. were given intraperitoneally either after preliminary metabolic rate determinations on the same day or to rats in which the basal level had been well established. The metabolic rates were then followed for 3 to 6 hours. In 9 instances there occurred definite increases in the heat production over the basal levels, ranging from 6 to 17% and with an average of 10.5%. This increase reached its maximum in 2 to 4 hours after the injection. In 2 experiments there was no apparent effect. Boiled extracts, in which the growth principle was destroyed, also caused an increased heat production of about the same magnitude (5 to 13%) in 4 rats. The extracts contained some protein and we believe that this increased metabolism may be due to the specific dynamic action of such protein products.

In 18 rats, 7 males and 11 females, frequent metabolic rate determinations were made before, during and after periods of daily administration of the extracts ranging from 15 to 72 days. In these experiments at least 24 hours elapsed after a dose of the extract before a metabolic rate determination was made. In 10 of the rats, 6 females and 4 males, there were significant decreases in the basal metabolic levels ranging from 12 to 39%. In 8 rats no significant effects were noted, but only one of these rats showed an increased growth rate and 4 received extracts which were found to be of slight potency. In no animal of the series was a significant increase in the basal metabolic level found.

There was a characteristic inertia in the metabolic response to the growth extracts in all of the rats showing a decrease. The fall did not begin until several days after the injections were started, and continued for 8 to 14 days after they were stopped. This lag is well illustrated by the female rat which showed the greatest response to the extracts. The average normal metabolic level in 8 determinations was 766 calories per day per square meter of body surface, with extreme ranges of 735 and 807 calories. Growth promoting extract was given for 19 days. During this period 6 determinations at 3 day intervals gave rates of 764, 728, 771, 640, 577 and 538

* These extracts were prepared and furnished by Parke, Davis & Co.

calories. After stopping the extract, the metabolic rate in 4 days fell to 470 calories and 5 days later it was found to have the same value. This represents a decrease of 39% from the original level. There was then a slow, gradual rise of the rate to a new level of about 740 calories, which was attained within 6 weeks. Although the response of this rat was greatest in magnitude, it was otherwise typical of the responses in the other rats.

5133

Differentiation of the in Vivo and in Vitro Actions of Tissue Extracts.

C. A. MILLS. With the assistance of Bernice Fick.

From the Laboratory for Experimental Medicine, University of Cincinnati.

In our earlier work[1, 2] on tissue extracts, we found them active in promoting clotting of the blood *in vitro* or when intravenously injected, and also when administered to animals or man subcutaneously, intraperitoneally or orally during fasting conditions. During those studies we were dealing with fresh extracts or freshly purified fractions. More recently we have noticed that certain batches of tissue fibrinogen, after weeks of storage, lose entirely their effectiveness on intraperitoneal injections into animals, although they retain their full activity *in vitro*. Other batches made apparently in the same manner, are active with all forms of administration.

This raises the question as to whether we are dealing with 2 different actions of tissue fibrinogen, one of which is destroyed on standing, or whether 2 separate coagulants are present, one acting only when added directly to blood, and the other capable of accelerating clotting after absorption into the blood through the intestine or peritoneum. We offer evidence here that there are 2 distinct substances of this nature existing in crude tissue extracts or fresh preparations of tissue fibrinogen.

Healthy rabbits of about 4 pounds body weight, and fasted for 24 hours, were used for the injection tests. Two rabbits were used for the testing of each sample and for control in each series. In

[1] Mills, C. A., *J. Biol. Chem.*, 1921, **46**, 135, 167.

[2] Mills, C. A., Dorst, S. E., Mynchenberg, G., and Nakayama, J., *Am. J. Physiol.*, 1923, **63**, 484.

cases of any considerable disagreement in the results for a given test, a second 2 rabbits were used in similar fashion. Blood was carefully withdrawn from the heart in oiled syringes just prior to the injections and again one hour afterward. One cc. portions of these blood samples were allowed to clot in clean Pyrex-Corning test tubes (1 cm. x 10 cm.) in a water bath at 40° C. Two such tubes were prepared at each bleeding, and thus checks were obtained on the reading of the clotting time. The rabbits were first accustomed to handling so that the heart puncture could be made without struggling, as it was found that even a few seconds of vigorous struggling or effort on the part of the rabbit greatly shortened the clotting time. The ingestion of food, or infection, had a similar effect.

In vitro tests for tissue fibrinogen activity were carried out in the usual way in citrated horse plasma.[3] The tissue fibrinogen consisted of the purified globulin fraction of fresh calf lung extracts.

Effects of exposure to ultra violet radiations were first studied, with a view to a possible use of this as a means of sterilizing the coagulant. Much to our surprise we found that even a few min-

TABLE I.
Effect of Ultra Violet Light on Tissue Fibrinogen.

Citrated plasma	Tissue fibrinogen	Irradiated for	$CaCl_2$ 1%	Clotting time
0.5 cc.			0.15 cc.	2 min. 40 sec.
" "	0.1 cc. in saline	0 min.	" "	30 "
" "	" " " "	5 "	" "	31 "
" "	" " " "	15 "	" "	30 "
" "	" " " "	30 "	" "	34 "
0.5 cc.	0.1 cc. in phosphate buffer	0 min.	0.15 cc.	34 sec.
" "	" " " " "	5 "	" "	36 "
" "	" " " " "	10 "	" "	36 "
" "	" " " " "	15 "	" "	32 "
" "	" " " " "	30 "	" "	38 "

Tested by Rabbit Injection

Tissue fibrinogen	Irradiated for	Reduction Clotting time 1 hour after injection
2 cc. in saline	0 min.	50%
	5 "	58%
	10 "	28%
	30 "	0%
2 cc. in phosphate buffer	0 min.	50%
	5 "	25%
	15 "	0%
	30 "	0%

[3] Mills, C. A., *J. Biol. Chem.*, 1921, **46**, 135.

utes exposure, at 15 inches distance, completely inactivated the solution so far as its effects after absorption into an animal were concerned. Ten cc. portions of the tissue fibrinogen (1.5% strength) in 0.9% NaCl solution, and in phosphate buffer at pH 7.1, were exposed in open sterile petri dishes at a distance of 15 inches from a mercury quartz tube for 5 to 30 minutes. These various portions were then tested for their coagulative activity *in vitro* and *in vivo,* with the results shown in Table I.

It is evident from the data in Table I that the irradiation up to 30 minutes does not produce any significant change in the *in vitro* activity of the tissue fibrinogen either in saline solution or in the phosphate buffer. However, the *in vivo* effectiveness is destroyed by the light within 30 minutes in the saline medium, and within 15 minutes in the phosphate buffer. These results would indicate beyond doubt that we are dealing either with 2 different substances, or with 2 separate functions of one compound.

Treatment of the tissue fibrinogen with various adsorbents gave further interesting information. Adsorbents used were Fuller's earth, Norite, and Lloyds' reagent. They were shaken with the tissue fibrinogen in saline solution and then centrifuged. The supernatant liquid was tested by injection into rabbits with the following results:

Fuller's earth,	fluid	opalescent,	29%	reduction in	clotting	time.	
Norite,	"	"	28%	"	"	"	"
Lloyd's reagent,							
(a) small amount,	"	"	59%	"	"	"	"
(b) in excess,	"	clear,	40%	"	"	"	"
Original tissue fibrinogen,	"	opalescent,	37%	"	"	"	"

With Lloyds' reagent we succeeded in ridding the tissue fibrinogen of all its suspended material and opalescence, leaving it watery clear, but with its effectiveness *in vivo* unimpaired. Treatment with a small amount of this reagent always served to increase the *in vivo* activity of the tissue fibrinogen, but there was always present some opalescence, although not nearly so much as in the original solution. These same solutions, tested for their *in vitro* activity, gave the following results:

		$CaCl_2$ 1%	Clotting Time
0.5 cc. plasma,	0.5 cc original tissue fibrinogen	0.15 cc	0′ 15″
" " "	" "	"	4′ 45″
" " "	" " supernatant liquid (Fuller's Earth)	"	0′ 50″
" " "	" " " (Norite)	"	0′ 28″
" " "	" " " (Lloyd's Reagent)		
	(a) (small amount)	"	1′ 25″
	(b) (in excess)	"	4′ 10″

With Lloyds' reagent we accomplished just the reverse of the ultraviolet light effect, that is, the factor effective *in vitro* is removed, while that effective *in vivo* is left unharmed, perhaps even increased in potency.

Boiling the tissue fibrinogen solution vigorously for a few minutes, and filtering, yielded a clear filtrate which was without effect on blood clotting *in vitro;* but which gave a marked inhibition of clotting when injected intraperitoneally.

We feel justified in concluding that tissue fibrinogen, as we previously prepared it, contains 2 substances, one of which is associated with the turbidity of the solution and acts as a strong blood coagulant only when added directly to the blood. The other can be obtained in a crystal clear solution, is without coagulative activity *in vitro* but acts well in the body. This latter substance is readily inactivated by ultraviolet light, while the former is resistant to such effects.

5134

Delayed Reproduction in Amblystoma Punctatum.

G. E. COGHILL AND M. T. CALDWELL.

From the Morris Biological Farm of the Wistar Institute of Anatomy and Biology.

Nine specimens of adult *Amblystoma punctatum,* captured in pools in the Pocono Mountains, near the village of Effort, Pennsylvania, during the breeding season (the first week of April) of 1929, were kept in the laboratory of the Morris Biological Farm of the Wistar Institute until February 14, 1930, when they were placed in a mechanical refrigerator. On May 28 two females and one male were found frozen to death. The others of the lot, one female and 5 males, were placed in an aquarium. On the morning of May 29 numerous spermathecae were found deposited on sphagnum in the aquarium, and during the night of May 29-30 two clutches of eggs were deposited. On the morning of May 31 another clutch, very small, was found, but it was probably deposited on the first night, for the eggs did not appear to differ from the others in degree of development.

The eggs were examined critically on June 7. There proved to be 125 in all. Seventy-six of these were developing normally in various neural groove and early tube stages (Harrison's stages 15 to 19);

36 were abnormal, mostly in an apparently abortive segmentation (possibly parthenogenetic); 11 were apparently infertile. The normal embryos progressed in development according to Harrison's stages approximately as follows: June 10, stage 26; June 11, stage 27; June 12, stage 31; June 13, stage 31+; June 15, stages 32 and 34; June 16, stage 34; June 17, stages 34 and 35; June 18, stages 35 and 36. On June 15, 9 were in the early flexure stage (Harrison's stages 33 and 34); 18 in the non-motile stage (Harrison's stages 33 and 34); the others were in the premotile stage. On June 16 almost all of the embryos were in various flexure stages; but none were in the coil stage. On June 17 several were in the coil stage. On June 18 the embryos were mostly in the S-reaction stage (Harrison's stage 36). On June 19 they were approaching closely to the swimming stage, and on June 20 (the time of writing) some were beginning to swim. The delayed reproduction has, therefore, not prevented normal structural and physiological development.

The parents of these embryos were kept from April, 1929, to February 14, 1930, in a tightly closed wooden box with a bedding of sphagnum but without water excepting as water was dashed over the sphagnum to keep it moist. The box in which they lived was kept in a moist place on the cement floor of a cellar and adjacent to a gutter of running spring water the temperature of which ranged during July and August from 12.4° C. to 14.6° C. During the remainder of the year it ran as low at 9.6° C. The temperature immediately about the specimens was probably a little above that of the water in the gutter. The food was almost exclusively earthworms which were thrown into the box without regard to individual feeding. The temperature of the refrigerator ranged from 33° F. to 36° F. except during defrosting, when it rose considerably, and on one occasion it fell below freezing.

The dimensions of the aquarium in which the eggs were deposited are 14x14x36 inches. It contained a large amount of sphagnum, and was supplied with a jet of compressed air, and a small stream of spring water which on the days concerned ran at a temperature of 11° C. The room temperature was 15.8° C.

Probably an important factor in the excitation of egg laying in this species under laboratory conditions is the presence of a number of males. Blanchard[1] has observed several males of *Amblystoma punctatum* swimming in a whorl around a single female at the time of egg-laying; and one of us has seen the same performance in the native breeding place.

[1] Blanchard, *Am. Naturalist*, 1930, **64**, 154.

5135

Specific Soluble Substances of the Pneumococcus in the Blood in Pneumonia.

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From the Biological Division of the Medical Clinic, the Johns Hopkins University and Hospital.

Avery and Dochez[1] detected the specific soluble substances of the pneumococcus in the urine of patients with pneumonia, and on the basis of their observations the determination of the type of pneumococcus causing the lesion in the lung may often be accomplished early in the course of the disease.

There is every reason to believe that the specific substances are brought to the kidney by the blood and concentrated in the urine. It is believed by some that the precipitin occurs in the urine only in cases with organisms in the blood. Our own observations have shown that at least the soluble antigen may be detected even where the blood culture is negative. It is of course possible that, as many believe, the pneumococci were present in the blood very early in the course of the disease and could not be found by blood culture when the patient came into the hospital. Since the specific soluble substances are so readily diffusible, it is not necessary to presuppose that organisms must be present in the blood when the soluble substances are detectable in the urine.

Since the soluble substances give a precipitate under appropriate conditions in high dilution, it might be possible to detect them early in the course of the disease and thus determine the type for serum treatment when specimens of sputum and urine are not obtained. But Blake,[2] using the untreated serum from patients with pneumonia, obtained precipitation by adding type specific antipneumococcus serum, only relatively late in the disease, on the sixth day. Out of 17 cases, he found the soluble substances in only 2 (one was a type II and the other Friedlander's bacillus infection).

By concentrating the ultra filtrate or the filtrate from coagulated patient's serum, the specific soluble substances in all 3 types have been found, and in one as early as the thirteenth hour after the initial chill.

The two methods in use at present are as follows:

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[1] Dochez, A. R., and Avery, O. T., *J. Exp. Med.*, 1917, **26**, 477.

[2] Blake, F. G., *Arch. Int. Med.*, 1918, **21**, 779.

Method A. Twenty cc. of the patient's blood are defibrinated by shaking with glass beads in an Erlenmeyer flask and centrifuged. The serum is placed in a Coor's filter impregnated with celloidin and centrifuged at high speed for 5 minutes (Toth's method[3]). Two cc. of sterile distilled water are added to the inside of the filter and the tube centrifuged again to wash through the filtrate which might be held in the filter. The collected filtrate is transferred to a test tube which is set in the steam bath and a bent glass tube connected to a vacuum pump is placed loosely in the test tube so that the open end of the bent tube does not quite touch the liquid. Rapid concentration of the filtrate to 3 cc. volume is accomplished in a few minutes. 0.25 cc. of the clear concentrated filtrate is added to each of 4 small tubes and 0.25 cc. of the type specific antipneumococcus serum is added. To the fourth tube containing filtrate, 0.24 cc. of salt solution is added for control. The tubes are placed in the 37° C. water bath and read in a beam of light at 5 minute intervals. In several instances precipitation has been observed after 5 minutes incubation.

The remainder of the filtrate (about 2 cc.) is used in a longer method of checking. To it 8 volumes of 95% alcohol are added and after mixing set in the ice-box over night. The flocculent precipitate is collected by centrifuging, discarding the supernatant fluid and drying the residue by heat, under a stream of air. 2 cc. of salt solution are added and after shaking is distributed to tubes for type differentiation.

Method B. To the serum collected as in Method A, are added 10 cc. of distilled water and 5 cc. of M/5 sodium acetate-acetic acid buffer pH 4.6 and the mixture boiled until coagulation is complete. The paper filtrate is evaporated to dryness over a free flame and heated carefully until the odor of acetic acid is no longer present. To the residue is added 5 cc. of sterile distilled water for extraction. The solution is either centrifuged or passed through a small paper filter and tested against the type specific sera as in Method A.

The results in 2 cases of type I, 4 cases of type II, and 1 case of type III have been checked either by sputum or by blood culture. In 6 cases the specific soluble substances were detected in blood when the blood culture remained negative. The method requires less time than the sputum culture or mouse method but has no advantage over the sputum extract method. It is useful for typing in cases in which neither sputum nor urine can be obtained.

The occurrence of specific soluble substances in the blood in cases

[3] Toth, A., *Biochem. Z.*, 1927, **191**, 355.

in which the blood cultures were negative is consistent with the fact that these substances are readily diffusible. Determinations of the specific soluble substances may have application in determining the amount of serum necessary and in following more closely the results of serum therapy.

5136

Effect of Tar on Experimental Teratoids in the Rat.

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Carrel,[1] White,[2] Fisher,[3] and others obtained metastasising sarcomata by injecting into fowls a mixture of embryo pulp and dilute arsenious acid or embryo in combination with tar. In a previous publication,[4] it was found that rat embryo combined with arsenic did not produce malignant growths in the rat, and so far as determined, the arsenic did not influence favorably the development of teratoids. In the present experiments, coal tar in various dilutions was combined with embryo pulp to determine its effect on teratoid growth.

From a thick ether extract of coal tar a 1-1000 stock dilution was made in a 5% gelatin and from this the various higher dilutions were made by mixing with one per cent gelatin. Usually about one-half an embryo was drawn directly into a syringe fitted with a needle having a 1-mm. lumen and in the syringe mixed with an equal volume of the tar dilution. The injections into the subcutaneous groin tissue followed immediately. The rats averaged three-quarters grown.

Several factors influence the development of teratoids. The genetic relationship of the animals no doubt, is an important variant in an in-bred strain of animals, such as the one used in these experiments. However, when a 1-500,000 strength of tar was used (experiment 9) all rats showed distinct teratoid growth. When the embryo pulp was mixed with 1-200,000 tar, the teratoid growth took place irregularly and often failed. With tar concentrations of

[1] Carrel, A., *Compt. rend. Soc. de Biol.*, 1925, **93**, 1083.

[2] White, A. W. M., *J. Cancer Res.*, 1927, **11**, iii.

[3] Fisher, A., *Compt. rend. Soc. de Biol.*, 1926, **94**, 1217.

[4] McJunkin, F. A., and Cikrit, M. F., PROC. SOC. EXP. BIOL. AND MED., 1929, **27**, 179.

TABLE I.

Experiment	No. of rats	Duration of Experiment	Treatment	Results
1	5	5 mo.	2-mm. embryo 1-200,000 tar	One rat has a 22x6x3 mm. teratoid; others negative.
2	6	35 days	4-mm. embryo 1-25,000 tar	No trace of growth.
3	6	52 "	4-mm. embryo 1-25,000 tar	" " " "
4	5	106 "	4-mm. embryo 1-200,000 tar	Two show hard 1-mm. granules; other 3 negative.
5	6	67 "	3-mm. embryo mixed with 1-200,000 tar. Also, 0-5 cc. 1-1000 tar (6 doses) into opposite groin	4 negative and 2 with minute teratoid granules
6	7	20 "	7-mm. embryo 1-60,000 tar	2 show indefinite thickenings in groin which microscopically contain teratoid tissue.
7	5	107 "	2-mm. embryo 1-200,000 tar. Also, 1 cc. 1-1000 tar was given intrapintoneal	2 with minute hard granules; 3 negative.
8	9	66 "	4-mm. embryo 1-25,000 tar. Intramuscular	No trace of teratoid growth.
9	7	31 "	10-mm. embryo 1-500,000 tar	All show growths readily palpable and distinct on dissection. Microscopically they consist of mixed epithelial and mesenchymal tissues. The largest is 4-mm.
10	7	128 "	2-mm. embryo 1-200,000 tar. Also, 1 cc. 1-1000 opposite groin	One with hard teratoid granule and others negative.
11	6	106 "	2-mm. embryo 1-200,000 intramuscular	5 negative; one animal lost.

1-25,000 (experiments 2, 3, and 8) no proliferation of the embryonic cells was demonstrable.

Embryonic rat cells brought into contact with tar have shown no tendency to undergo malignant transformations. The only influence observed was an inhibition of teratoid development by the tar in the greater concentrations.

5137

Effects on the Optic Lobes and Nerve Cells of Opticus Layer of Experimental Extirpation of One Eye in Larvae of the Tree-Frog (Hyla regilla).

O. LARSELL.

From the Anatomical Laboratory, University of Oregon Medical School, Portland.

Frog larvae of 18 to 22 mm. total length were operated by excising the left eye. Optic function had been established some time previously, as indicated by optic reflexes, and optic fibers had grown into the opticus layer, as shown by these reflexes and by histological preparations of pre-operative stages. The operated larvae were fixed at metamorphosis, and were stained by the Golgi method and other methods best adapted to bring out nerve cells and their processes. As previously reported,[1] there was a marked reduction in size of the affected (right) optic lobe, and a reduced number of cells in the 8th and 9th layers. The present study has added the facts that the cells of the opticus layer do not attain the same size as corresponding cells of the opposite side and their dendritic processes fail to show normal development.

These results appear due to the absence of the normal optic stimuli which reach the opticus layer in the unoperated larva, or to the absence of metabolic or other factors directly attributable to these stimuli. The fibers of the deeper layers of the optic lobe were not involved, since the optic fibers, which were destroyed by enucleation, reach only the outermost layer (9th or opticus) and the 7th layer. Nevertheless there was reduced proliferation of cells, which develop in the deep part of the lobe, with resulting hypoplasia of the entire lobe, as well as the incomplete development of the nerve cells in the part directly affected.

[1] Larsell, O., *J. Comp. Neur.*, 1929, **48**, 331.

5138

Studies on the Nature of the Viricidal Antibodies in Anti-Poliomyelitis Serum.*

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From the Department of Bacteriology and Experimental Pathology, Stanford University, California.

That poliomyelitis convalescent serum, whether of human or monkey origin, possesses viricidal properties demonstrable *in vitro* is a well established fact (Levaditi and Landsteiner,[1] Flexner and Lewis,[2] Netter and Levaditi,[3] and many others). The nature of the antibodies responsible for the inactivation of the virus has not been determined. While the results of several investigators (Lebredo and Recio,[4] Neustaedter and Banzhaf[5]) suggest that they may be cytolytic or bactericidal (complement fixing) in nature, others (Wollstein,[6] Gay and Lucas,[7] Römer and Joseph[8]) have been entirely unable to elicit evidence of specific antibodies of this type. Because of the difference in the results reported, particularly when considered in the light of the ultrafiltrable nature of the virus (Krueger and Schultz[9]), a careful study has been undertaken to determine, if possible, the exact nature of the antibodies formed against this virus. The work has been carried out with both human and monkey convalescent serum.

No difficulty has been experienced in demonstrating viricidal antibodies in convalescent sera tested *in vitro* in the customary manner. *No evidence of the presence of specific complement fixing and precipitating antibodies for the virus could, however, be elicited in any of these sera.* These results harmonize with those in earlier work on vaccinia, rabies, herpes and the bacteriophage (Schultz *et al*[10]). To determine further the relationship of the antibodies responsible for

* These studies were supported by Mrs. John W. Mitchell and the Mary Hooper Somers Medical Research Fund.

[1] Levaditi and Landsteiner, *Compt. rend. Soc. biol.*, 1910, **68**, 311.

[2] Flexner and Lewis, *J. Am. Med. Assn.*, 1910, **54**, 1780.

[3] Netter and Levaditi, *Compt. rend. Soc. biol.*, 1910, **68**, 617.

[4] Lebredo and Recio, *Sanidad y Beneficiencia* (Habana), 1910, **3**, 170. Cited by Gay and Lucas, *Arch. Int. Med.*, 1910.

[5] Neustaedter and Banzhaf, *J. Infect. Dis.*, 1917, **21**, 515.

[6] Wollstein, *J. Exp. Med.*, 1908, **10**, 476.

[7] Gay and Lucas, *Arch. Int. Med.*, 1910, **6**, 330.

[8] Römer and Joseph, *Münch. med. Wochenschr.*, 1910, **57**, 945.

[9] Krueger and Schultz, PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 600.

[10] Schultz, *et al.*, *J. Immunol.*, 1928, **15**, 229; 243; 265; 411; 1929, **17**, 245.

the inactivation of the virus, tests were carried out to determine whether neutral serum-virus mixtures are *dissociable,* and whether *time* is a factor in the inactivation of virus by immune serum. The results have been in the affirmative.

Experiment 1. *To Determine Whether Neutral Serum-Virus Mixtures are Dissociable.*

Four monkeys were used. Two were injected with undiluted serum-virus mixture, administered after the usual 2-hour incubation at 37° C., while 2 received corresponding mixtures diluted with 9 volumes of physiological saline immediately before injection. One monkey in each set of 2 was injected with normal serum-virus mixtures, undiluted and diluted, for control purposes. The results show that a neutral serum-virus mixture may be rendered infective again by the addition of a suitable volume of physiological saline. These observations, which agree with those made on neutral toxin-antitoxin mixtures by other investigators (Madsen,[11] Otto and Sachs,[12] Glenny[13]) indicate clearly that the inactivation of poliomyelitis virus by immune serum resembles more closely a toxin-antitoxin reaction than a bactericidal type of reaction.

Experiment 2. *Influence of Time on the Neutralization of the Virus by Immune Serum.*

Three groups of 2 monkeys each were inoculated with serum-virus mixtures which had been incubated for different lengths of time. One monkey in each set, receiving normal serum-virus mixtures, served as controls. The first set of 2 animals received the serum-virus mixtures immediately after the 2 reagents were brought together, the second set of 2 animals received similar mixtures after these had been incubated at 37° C. for 24 hours and the third set of 2 received mixtures after 48 hours' incubation. Of these 6 animals, only the 2 receiving immune serum-virus mixtures incubated 24 and 48 hours respectively, escaped the experimental disease. These results seem to indicate that *time* plays an important rôle in the inactivation.

In carrying out this experiment 2 precautions were regarded essential, one relating to the dilution of the immune serum to be used, the other to the prevention of spontaneous deterioration of the virus incident to prolonged incubation at 37° C. That the dilution of an immune serum may conceivably influence the rate of inactivation seems obvious, though this is sometimes overlooked. A concentrated

[11] Madsen, *Centralbl. f. Bakt.*, 1906, Orig., **37**, 373.

[12] Otto and Sachs, *Z. f. Exp. Path.*, 1906, **3**, 19.

[13] Glenny, *J. Hyg.*, 1925, **24**, 301.

immune serum added to an appropriate quantity of virus may render such a virus suspension innocuous immediately after the serum is added. In harmony with the results of other investigators with certain other viruses, we have repeatedly injected monkeys with poliomyelitis virus treated with *undiluted* monkey poliomyelitis convalescent serum immediately before inoculation (that is, without any incubation of the mixtures) without realizing infection. These observations have given rise to the opinion that the immune sera against the viruses may not act directly on the viruses, but in some indirect manner change the susceptibility of the tissues of the host. However, the fact that with dilution of the immune serum, it becomes necessary to prolong its contact with the virus *in vitro* argues distinctly in favor of the opinion that the immune serum *does* act directly on the virus. The relationship between the factor of *time* and that of *dilution* is brought out clearly by Schultz, Quigley and Bullock[14] on the inactivation of bacteriophage. They noted that whereas a serum dilution of 1:128 served to inactivate a given bacteriophage suspension in 30 minutes, a serum dilution of 1:4098 required as long as 8 days to render the same bacteriophage suspension inactive. It appears safe to assume that essentially the same relationships may apply to poliomyelitis and other virus-serum mixtures.

5139

Immune Serum Production in Poliomyelitis Refractory Animals.*

E. W. SCHULTZ AND L. P. GEBHARDT.

From the Department of Bacteriology and Experimental Pathology, Stanford University, California.

Several investigators have reported the production of antipoliomyelitis serum in poliomyelitis refractory animals. Thus, Kraus[1] and likewise Pettit[2] claim to have been able to produce a viricidal serum in sheep, while more recently Neustaedter and Banzhaf[3]

[14] Schultz, Quigley and Bullock, *J. Immunol.*, 1929, **17**, 245.

* These studies were supported by Mrs. John W. Mitchell and the Mary Hooper Somers Medical Research Fund.

[1] Kraus, *Z. f. Immunitätsforsch. u. Exp. Therapie*, 1911, **9**, 117.

[2] Pettit, *Compt. rend. Soc. biol.*, 1918, **81**, 1087.

[3] Neustaedter and Banzhaf, *J. Am. Med. Assn.*, 1917, **68**, 1531.

Pettit,[4] Weyer, Park and Banzhaf,[5] and Fairbrother[6] have reported similar results with the serum of horses immunized with virus material from monkeys. The observations of other investigators (Flexner,[7] Stewart and Haselbauer[8]) have not supported the view that refractory animals are serviceable for the production of antipoliomyelitis serum. We began in 1928 to make a careful study of the capacity of various refractory animals to produce viricidal sera against this virus. The animals used include the guinea pig, rabbit, dog, goat, sheep and horse. These animals received injections of virus material (ground cord and medulla) from poliomyelitis monkeys, administered by various routes, at short intervals of time over periods ranging from several months for some of the animals to more than a year for others (sheep). The sera of these animals have been tested for viricidal properties in the customary manner, except that we aimed to subject the supposedly immune sera to somewhat more severe tests than those described by previous workers. Our method in setting up the viricidal test has been to add to a given volume (1 cc.) of a 5% suspension of virus cord (very finely ground in a machine,[9] lightly centrifuged and filtered through filter paper) to an equal volume (rather than 9 times the volume) of the undiluted immune serum. This mixture was incubated for 2 hours at 37° C. and injected in doses of 1.5 cc. into the frontal lobe of the brain of a monkey. Certain of the immune sera so produced were compared with monkey convalescent serum by titrating them against the same virus suspension. In several of the tests carried out with *undiluted* immune rabbit, sheep, and horse serum, a well defined viricidal action was obtained, but *on diluting these sera, even to a dilution of* 1:2 (after addition of virus suspension, 1:4), *no viricidal action was realized with any of these sera.* A convalescent monkey serum, on the other hand, exercised a viricidal action in dilutions as high as 1:64 under the same conditions. All the tests were controlled with corresponding set-ups made with normal sera. Our results thus far, therefore, indicate that refractory animals are incapable of producing antisera comparable in viricidal activity to monkey convalescent serum.

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[4] Pettit, *Bull. gén. de therap.*, 1925, **176**, 389.
[5] Weyer, Park and Banzhaf, *Am. J. Path.*, 1929, **5**, 517.
[6] Fairbrother, *Brit. J. Exp. Path.*, 1930, **9**, 43.
[7] Flexner, *Lancet*, 1912, **2**, 1271.
[8] Stewart and Haselbauer, *J. Exp. Med.*, 1928, **48**, 449.
[9] Schultz and Banham, *Am. J. Publ. Health*, 1930, **20**, 771.

5140

A Method for the Purification of the Bacteriophage.

C. E. CLIFTON. (Introduced by E. W. Schultz.)

From the Department of Bacteriology and Experimental Pathology, Stanford University, California.

Numerous observers have reported the adsorption of bacteriophage corpuscles by both electro-positive and electro-negative colloids. D'Herelle[1] in a review of the literature concludes that in an alkaline medium the bacteriophage is adsorbed only by electro-positive colloids and therefore carries a negative charge, a conclusion in agreement with cataphoresis studies.

De Necker[2] reported the partial flocculation of the bacteriophage by aluminum hydroxide, an electro-positive sol. When the alumina mass is dissolved by the careful addition of acetic acid the bacteriophage reappears in the liquid. Sommer, Sommer, and Meyer[3] reported the isolation of botulinus toxin by selective adsorption on colloidal aluminum hydroxide, elution with secondary ammonium phosphate, removal of salts by dialysis, and evaporation to dryness at 40° C. Their method was used in the following attempt to purify the bacteriophage.

When a suspension of the staphylococcus bacteriophage in Martin's broth (pH 7.8) is stirred with an equal volume of a 3.5% suspension of aluminum hydroxide for one hour the bacteriophage is completely removed from the mother liquor. After washing the precipitate 4 to 6 times with distilled water no bacteriophage corpuscles can be detected in the final wash water. When the precipitate is triturated with a 0.5% solution of secondary ammonium phosphate (pH 8.0) the bacteriophage is liberated and can be separated from the alumina by centrifugation or filtration.

The final procedure adopted is as follows: 50 cc. of a suspension of the staphylococcus bacteriophage in Martin's broth is stirred for one hour with an equal volume of the aluminum hydroxide suspension. This is collected on a Buchner funnel and washed 5 times with distilled water. The washed alumina mass is stirred for ½ hour with 50 cc. of the secondary ammonium phosphate solution and then placed in the 32° C. incubator over night. The alumina is fil-

[1] D'Herelle, "The Bacteriophage and its Behavior," Williams and Wilkins, Baltimore, 1926.

[2] De Necker, *Compt. rend. Soc. de Biol.*, 1922, **87**, 1247.

[3] Sommer, Sommer, and Meyer, *J. Infect. Dis.*, 1926, **39**, 345.

tered off by suction and the bacteriophage is recovered in the filtrate.

The original bacteriophage suspension was active in a dilution of 10^{-9}. No activity could be detected in the original filtrate or in the final wash-water but after elution the purified phage was active in a dilution of 10^{-7}. The ordinary color tests for proteins were negative. No precipitate was formed by ½ or complete saturation with ammonium sulfate.

Further purification studies by means of selective adsorption are now in progress and also attempts to remove salts by dialysis and to concentrate the suspension *in vacuo*.

5141

Capillary Circulation in Skeletal Muscle in Rest and in Exercise.

E. G. MARTIN, EOLA WOOLLEY AND MIRIAM MILLER.

From the Laboratory of Physiology, Stanford University.

Method—Gracilis muscles in anesthetized dogs were injected with India ink introduced under pressure centrally into the femoral artery below the origin of the branch artery to the muscle without interrupting the circulation. The injection was continued until the venous blood showed complete blackening. The muscle was quickly clamped off, excised, and dropped at once into Bouin's fluid for fixation. Paraffin sections about 10 to 15μ thick were made for counting. To study the effect of exercise, a muscle was stimulated directly through the obturator nerve by means of brief tetani repeated about once a second for about 3 to 5 minutes. India ink was injected while the muscle was being stimulated.

Results on muscles of amytalized dogs. In resting gracilis about 925 capillaries were injected per sq. mm. of tissue. These are figures for injected bundles. There were also many bundles in which larger vessels contained ink but capillaries none. In exercised gracilis about 1900 capillaries were injected per sq. mm. tissue. An occasional uninjected bundle was seen in these muscles, but far fewer than in resting muscles.

$$\text{Ratio } \frac{1900}{925} = 2.05$$

The maximum number of capillaries per sq. mm. (about 2200) was seen in muscles injected ½ hour after death. No uninjected bundles were seen.

Results on muscles of dogs anesthetized with morphine and ether. The technique was the same as above except for the method of counting. In these experiments the number of open capillaries was compared with the number of muscle fibers in a field of given area. In resting muscle the results were: fibers 4.40; capillaries 2.65, or 0.60 capillary per fiber. In active muscle the results were: fibers 4.44; capillaries 5.65, or 1.27 capillary per fiber.

$$\text{Ratio } \frac{1.27}{0.60} = 2.11$$

These results appear to correspond more nearly with the findings of Hartman, Evans and Walker[1] than with the earlier observations reported by Krogh.[2] The choice of anesthetic appears to have made no difference in these experiments in the number of capillaries open in rest and in exercise.

5142

Certain Physiological Changes Accompanying Prolonged Mental Reaction

MARY L. SMULL, A. S. RAUBENHEIMER, F. M. BALDWIN, RALPH WEBB, EDITH HARKER, ZELMA HUXTABLE AND MARJORIE ABERNETHY. (Introduced by G. Martin.)

From the Department of Zoology, University of Southern California.

In an attempt to note the extent and to find the limits of certain suspected physiological changes produced during periods of prolonged mental effort, several series of carefully planned experiments were undertaken involving 3 subjects familiar with the solution of mental arithmetic problems. The task was uniform throughout and consisted in the solution of multiplication of 4 numbers by 4 numbers from memory alone. The level of metabolic rate was measured by oxygen consumption using the Graphic Metabolism machine. Changes in the blood elements, erythrocytes and leucocytes and changes in hemoglobin content were checked in the usual manner by use of the hemocytometer and the hemoglobinometer. Conspicuous changes in excretory products were determined by routine urine analyses, involving variations in specific gravity, reaction, occur-

[1] Hartman, F. A., Evans, J. I., and Walker, H. G., *Am. J. Physiol.*, 1929, **90**, 668.

[2] Krogh, A., "The Anatomy and Physiology of Capillaries," New Haven, 1922.

rence of albumin and sugar, di-acetic acid and acetone. Five specific tests were made on each of the subjects, the first series to establish the "norms" without mental effort. The second series covered a one-hour concentration period. The succeeding tests were for 4, 8, and 12 hours duration, respectively, repeated on 4 successive days. Checks were made preceding and following the 4-day series to establish metabolic levels. In general there appears a slight rise in the metabolic level on the second day of the 12-hour series in 2 of the 3 subjects. On the third day the metabolic level of all 3 continued well above the basal rate, while records from the fourth day show an extremely high level in all subjects. The urine analysis showed fluctuations in specific gravity within normal limits; the reaction was from neutral to acid during the daily interval with no trace of either sugar or albumin in any subject. Acetone was positive after the first day, but with no traces of di-acetic acid. There was no apparent significant change in the hemoglobin content of the blood, and although the erythrocyte and the leucocyte counts show that these elements in the blood increase in numbers during the day these fluctuations are considered to be within normal limits when one day is compared with another throughout the 4-day cycles.

5143

A Simple Spirometer.

FRANCIS MARSH BALDWIN. (Introduced by E. G. Martin.)

From the Department of Zoology, University of Southern California.

In attempting to carry out the laboratory exercises on the volumetric changes in respiration one often finds large sections of elementary students in classes with perhaps a single rather high priced and somewhat delicate piece of apparatus—the Spirometer. A rather simple and seemingly sufficiently accurate piece has been devised which can easily be assembled by appropriating glassware found at hand.

Two large battery jars, 10 liters capacity, are filled two-thirds full of tap water taking care that the level of the water is the same in each jar. A 1000 cc. graduate cylinder is also filled with water and inverted in one of the jars, taking care that no air is allowed to enter on inversion. The respired gasses to be measured are collected by displacement. The delivery tube for the purpose is assembled and

consists of a glass mouth-piece of 10 mm. bore of convenient length connected to some length of rubber tubing, which in turn is joined to a 'Y' tube of the same size. The 2 tubes of the Y are each connected by rubber tubing to other Y tubes of approximately 7 mm. bore. A third series of Y tubes of approximately 4 mm. bore serve to attach the delivery tubes of 2 mm. bore and which are approximately 30 cm. long. By immersing 8 small delivery tubes to the bottom of the partially filled battery jars and by blowing forcefully as in testing vital capacity in the usual way the gas from one tube is collected in the graduate cylinder. Assuming that each tube delivers approximately one-eighth of the total gas escapement in the experiment the total volume can easily be computed.

The coefficients of correlation found between results in this spirometer and results in the Tyces and Sanborn commercial spirometer are +0.85 and +0.88 respectively, computed on the basis of 154 cases.

5144

Effects of Total Absence of Function on the Optic System of Rabbits.

LOUIS GOODMAN. (Introduced by W. F. Allen.)

From the Anatomy Department of the University of Oregon Medical School.

Despite numerous experiments on the various morphogenetic factors involved in the development of the central nervous system, the effect of normal physiological stimulation of an end organ on that end organ and on the corresponding brain centers is still little understood. The rôle of function in post-embryonic growth and differentiation of an established functional nerve tract in mammals has received but slight attention. In regard to the visual apparatus, 2 procedures have been used—extirpation of eyes, and sewing of eyelids. The results of extirpation on the visual cortex vary with the animal used, its age, and the experimenter; but the effects on the optic nerve and superior colliculus are uniform. The interpretation of such results from standpoint of lack of function is physiologically unsound as there are complicating factors of traumatic and secondary degeneration, and Von Gudden's degeneration. The results of sewing of eyelids likewise vary with the animal and the experimenter. Whereas Von Gudden noticed no changes in the cerebral cortices of rabbits whose eyes had been enucleated or eyelids sewn at birth,

Berger (1900), in artificial ankyloblepharon in cats and dogs found no macroscopic or cytological changes in any part of the optic system except the visual cortex, where he noticed an arrested development both grossly and microscopically. This he attributed to reduced function, though he admitted that all light was not excluded, and suggested that atrophy of the optic nerves and lower reflex centers would probably have also occurred if all light had been excluded. Not much advance has been made since.

A technic is required which removes function completely, allows the tract under investigation to remain anatomically intact, and permits absence of function to be the only variable. Rabbits were born in a dark room of special construction. The total absence of light was proven by exposure of panchromatic films for hours. When developed, these films showed no clouding whatsoever. The diet and cleanliness of the animals were carefully tended. Rabbits were removed at monthly intervals, the last group being 6 months old, and their weight, size, eyes, and brains carefully compared with their specific controls. The animals were born and killed in the dark, and at no time have they seen light. The experiments were not only controlled by normals, but also by enucleation and lid sewing experiments. The left eye was removed or lid closed, the unoperated side acting as control in each instance (there is almost complete decussation in the rabbit). Some of these animals were reared in the light, and others in the dark room along with the rabbits comprising the main experiment. In this way, then, the results of enucleation, sewing, and total absence of function could be compared.

The present report deals with the gross results of the first 3 months of the dark room experiments. The rabbits showed excellent growth when compared with their controls. The eyes, optic nerves, superior colliculi, lateral geniculate bodies, and cerebral cortices showed by careful observation and measurements, no differences not as easily demonstrated in any unselected group of normal controls of the same age and weight. The cortex of the experimental rabbit removed at 3 months could be differentiated from that of its particular control, but that this differentiation was not based on any fundamental alterations in structure is indicated by the fact that when the experimental brain was placed in a series of 8 normal rabbit brains of the same or greater age, it could not be picked out. The striking difference between the results of extirpation of an eye and the removal of function may be illustrated: One of the dark room animals had its left eye excised on the tenth day after birth, and was killed at one month. The cerebral stump of the left

optic nerve, and the right optic tract and colliculus showed the typical Von Gudden picture of degeneration. The right unoperated optic nerve, and left tract and superior colliculus, which were anatomically intact but had never functioned, could not be differentiated from a normal control of the same age reared in the light. The occipital regions at this stage showed no alterations, nor could the 2 cortices be told from those of the control. This shows strikingly the difference between the 2 technics, and points toward the invalidity of removal of an end organ as a physiological method of inquiry into the effects of lack of function on the neurones of the second and third order.

5145

The Question of Sex Hormone Antagonism.*

CARL R. MOORE AND DOROTHY PRICE. (Introduced by B. H. Willier.)

From the Hull Zoological Laboratory, the University of Chicago.

The preparation of active hormone substances in the Department of Physiological Chemistry and Pharmacology and the development of testis hormone detection methods for the laboratory mammal in the Department of Zoology has enabled the Chicago group[1] during the last 4 years to study more directly, by injections, the effects of separate or combined sex hormone action in the rat.†

The results here given have been the basis for a new conception regarding the interaction of hypophysial and gonadal secretions in the organism, which effectively removes the troublesome notion of sex hormone antagonism as an interpretation.

Injections into the castrated male gave results upon the male ac-

* This investigation has been aided by a grant from the Committee on Research in Problems of Sex of the National Research Council; grant administered by F. R. Lillie.

[1] For a review of our publications on this work see papers by Gallagher and Koch, *J. Biol. Chem.*, 1929, **84**, 495; Moore and collaborators, *Am. J. Anat.*, 1930, **45**, Gallagher and Koch, Moore and Gallagher, *J. Phar. and Exp. Therap.*, in press.

† But for the generous cooperation of members of the department of Physiological Chemistry and Pharmacology in providing us with active preparations for biological study, this paper could not have been written. We delight in making acknowledgements to T. F. Gallagher and F. C. Koch for testis hormone; to R. G. Gustavson and F. D'Amour for oestrin; to H. B. VanDyke for hypophysis hormone; and to L. T. Samuels for animals maintained upon vitamin B deficient, and inanition, diets.

cessories (prostate gland and seminal vesicles) as follows: I. Oestrin—no effect; II, testis hormone—normal accessories; III, testis hormone + oestrin—normal accessories. No antagonism is evident with mixtures of gonad hormones. Injections into normal males gave IV, oestrin—testicular damage, castrate accessories; V, oestrin + testis hormone—testicular damage, normal accessories; VI, brain, heart or liver lipoids—normal or damaged testes and normal accessories. VII, Cryptorchidism caused marked testis damage but normal accessories.

We have advanced the working hypothesis that gonadal hormones suppress the hypophysis, making available to the organism a reduced amount of gonadal stimulating secretion; that hypophysis hormone is necessary for gametogenesis or hormone production by the gonads; that sex hormones act directly upon homologous accessories, with or without the presence of hypophysis secretion, and have no effect upon heterologous accessories; and that sex hormones have no direct action upon the gonads.

Applying this interpretation to the above mentioned results, (I) oestrin is without effect upon male accessories. (II) Testis hormone stimulates male accessories. (III) Oestrin is neither chemically nor biologically antagonistic to testis hormone in castrated males. (IV) Oestrin suppresses the hypophysis making unavailable the secretion necessary for gonadal function hence gametogenetic damage and lack of hormone production; without testis hormone accessories are castrate. The addition of testis hormone as in (V) rectifies the hormone loss but not gametogenetic damage. Gametogenetic damage as in (VI) or (VII) does not involve interference with hormone production hence accessories are normal.

The hypothesis also accounts logically for other observations made in this laboratory as follows: VIII 30-day-old males injected with oestrin experience testicular depression and castrate accessories. Here oestrin suppresses the hypophysis; hypophysial sex stimulating secretions are reduced; testis growth and endocrine function are inhibited and without the latter, accessories are castrate. IX 30-day-old males injected with testis hormone experience testicular depression but have normal accessories. Hypophysis secretion is again affected and testis growth suppressed but the injected testis hormone acts directly upon the accessories, consequently they are normal or precociously stimulated. X Fresh hypophysial implants or hypophysis hormone precociously matures young males since secretions introduced by either means act through the testis.

XI Hypophysectomy leads to testis degeneration and castrate accessories but addition of testis hormone rebuilds the castrate accessories without repairing testis damage. XII Testis hormone introduction into normal females suppresses oestrus cycles and addition of hypophysis extract returns the cycle. Here, testis hormone is believed to suppress hypophysis secretion necessary for ovarian control and cycles cease; replacement of the secretion by hypophysis hormone injection brings on a new cycle due to renewed ovarian activity. XIII Oestrin injected into normal males produces testis damage and loss of hormone production (IV) but additions of fresh hypophysial implants or hypophysis hormone repairs the damage and leaves normal accessories. Hypophysial suppression by oestrin is therefore counteracted by introduction of its secretion and normal conditions follow. XIV Injurious effects of testis hormone injection into young males (IX) are overcome by hypophysial secretion administration. XV Testes of animals maintained upon vitamin B deficient diets have normal seminiferous tubules but accessories castrate in type. Here, hypophysial secretions are believed to be low in response to a low nutritive state and not as a specific response to vitamin B absence. Introduction of male hormone or of hypophysis secretion replaces castrate accessories by normal ones. XVI Maintenance upon inanition diets containing excessive vitamin B requirements gives normal testes and castrate accessories. Absence of vitamin B is not specific but a lowered hypophysis secretion is believed to be the cause.

The working hypothesis outlined above has been productive in suggestions for further work and our own results as well as those of many others, find in it a ready explanation. With advances in our knowledge, modifications or additions will undoubtedly have to be made. The conflicting results when interpreted upon an antagonism basis, are here found to be entirely consistent.

5146

Effect of Certain Endocrine Secretions on the X Zone of the Adrenal Cortex of the Mouse.*

STEVEN J. MARTIN. (Introduced by Frederick L. Hisaw.)

From Zoology Department, University of Wisconsin.

The discovery of a sex dimorphism in the adrenal cortex of the young mouse[1] and its relation to age and sex[2] suggests that this structure is influenced by certain endocrine secretions. The dimorphism, termed the X zone by Miller,[2] is present in the female adrenal and lies between the *zona fasciculata* and the medulla, the cells at this border being freely interspersed among each other; in the male adrenal, the X zone is absent and the *zona fasciculata* and the medulla are clearly separated by a distinct connective tissue capsule. This morphological difference is definitely established in mice 40 days of age. An attempt was made in this investigation to determine the effect upon the X zone of injecting various hormones which are intimately related to sex.

The procedure employed in this research involved the extraction and standardization of 5 endocrines, the daily injection of these preparations into normal and castrate mice of varying ages and finally the histological study of stained sections of the adrenal glands. A total of 155 mice were used, of which 79 were injected with different hormones, 56 served as controls and the remaining 20 were employed in testing extracts. The results may be conveniently summarized as follows:

The presence of the X zone in the adrenal gland of male and female mice 21 days of age and the establishment of a sex dimorphism in this gland at 40 days by its disappearance in the male adrenal have been confirmed. The characteristic persistence of the zone in castrate males and the absence of an effect following ovariectomy in females have also been confirmed.

The body weight was not affected by the continued injection of the 5 preparations employed. It does not seem plausible to ascribe the changes produced in the adrenal cortex of the mouse to any toxic action of the injected material.

*Assisted by grants from the National Research Council, Committee on Problems of Sex, administered by Dr. Frederick L. Hisaw.

[1] Masui, K., and Tamura, Y., *J. Coll. of Agriculture, Imperial Univ. of Tokyo*, 1926, **7**, 353.

[2] Miller, E. H., *Am. J. of Anat.*, 1928, **39**, 251.

The injection of standardized preparations of oestrin, corpus luteum, testicular hormone and the lutelizing and gonad stimulating hormones of the anterior hypophysis, for short periods (3-8 days) produced no change in the character of the X zone.

Injection of the corpus luteum hormone (oestrin free) which is responsible for the inhibition of oestrum, the vacuolation of the vaginal mucosa, production of placentomata and the pseudopregnancy picture in the rabbit's uterus, for either long or short periods (4 to 30 days) did not change the sex dimorphism in the adrenal glands of normal and castrate male and female mice.

Total degeneration of the X zone was produced by prolonged injection of oestrin into immature castrate males and normal and spayed females. In corresponding adult animals, marked but incomplete atrophy was found in the adrenals. In normal immature males, continued injections of oestrin caused a persistence of the zone or a feminization of the male character of the adrenal gland. In the normal adult male, the X zone reappeared upon administration of oestrin.

Injections of the lutelizing hormone of the anterior hypophysis for short or extended periods had no effect on the X zone. The result following prolonged injections of the gonad stimulating hormone of the anterior hypophysis tended to show that the X zone increased in size in the immature normal and spayed female and that it persisted in the immature normal male whose testicles remained normal.

An extended period of injection of the testicular hormone into mice that possess an X zone, produced a uniform effect in that a total or marked disappearance resulted.

It is suggested that in the non-castrate male mouse, the X zone is inhibited by the presence of the testicular hormone and that in the castrate male, normal and spayed female, its presence is dependent upon the gonad stimulating hormone.

5147

Excretion of Urea in the Rabbit at Different Age Periods.*

K. S. CHOUKE. (Introduced by F. E. Chidester.)

From the Department of Anatomy, University of Colorado School of Medicine, Denver.

Urea determinations have been made in many kinds of animals and under various conditions, but the literature apparently does not contain any reference to a relationship of urea excretion in normal rabbits to the age of the animal. This investigation was therefore undertaken to ascertain the daily amount of urea nitrogen excreted in the urine of rabbits from birth to adult condition. Fifty-one rabbits were used, including all ages from fully grown fetuses to adult animals.

Collection of urine. To collect the urine from the fetuses the mother was killed by decapitation, and the abdomen of the fetus after removal from the uterus was opened by an incision. The urine was removed by means of a fine hypodermic needle. The same method was used for collecting urine from the new born rabbits up to the age of 4 days. In these experiments practically no urine was found in the bladders of 8 fetuses and 12 new born rabbits, 2 to 5 minutes after parturition, except for a very small drop occasionally encountered, which was not sufficient to make urea determination by the procedure employed. Dr. Wallin† informs me that he has made similar observations on the absence of urine in the bladder of fetuses or new born rabbits. It is interesting to note that whenever the new born rabbits were permitted to feed, the bladder was seldom, if ever, found to be full. On the other hand, if they were taken away from the mother at birth and starved for from 10 hours to 3 days, the bladder on opening was almost always full of urine.

In one set of experiments animals over 10 days old were kept in metabolism cages, the urine being collected in a beaker placed underneath, and changed every 24 hours. No attempt was made to squeeze out the urine from the bladder. The animals were fed hay (dried alfalfa), fresh lettuce, celery, grass, rolled barley, and water *ad lib.* The younger animals were fed milk, with the additions of bread crumbs in some cases.

* An abstract of this paper was presented before the American Association of Anatomists at Rochester, March, 1929.

† Wallin, I. E., personal communication.

In one experiment an animal from the age of 26 days to that of 98 days was kept on a special diet consisting of measured amounts of milk, sucrose, and dried alfalfa.

In a second experiment 2 litter mates were maintained at a constant body weight while they were advancing in age. Since one of these animals, Rabbit R., died of starvation, the other was permitted to gain somewhat in weight to keep it alive. No record was kept of the amount of food consumed.

In the third experiment 3 litter mates were used, 2 of which received a special diet consisting of measured amounts of crushed barley, dried alfalfa and fresh lettuce, the third, control animal, receiving the same foods *ad lib.*, from the age of 43 days to that of 92 days.

The object was to maintain the weight of the 2 animals constant by giving just enough food to keep them alive. Actually the animals were allowed to gain about 100 gm. each in the course of the experiment, because previous experience had shown that otherwise the animals would die of starvation.

Determination of urea. Urea was determined according to the method of Folin and Youngburg[1] by direct Nesslerization.

From an analysis of the results it is evident that the amount of urea in the urine increases with the age as well as with the weight of the animal, from 1.5 mgm. at the age of 6 hours to about 1200 mgm. in the adult. The concentration of urea in the urine was variable.

Diet seems to have a definite influence on the amount of urea in the urine, the urea increasing on diets rich in proteins and certain amino acids. The decrease in urea nitrogen on changing to a diet poor in proteins was striking. In one instance it fell from 260 mgm. to 53 mgm. in one day and continued at a low level throughout the period of this low protein diet. The increase in urea nitrogen after changing the diet from the low protein to the ordinary diet was slow but definite.

The volume of urine decreased from 96 cc. to 6.6 cc. per 24 hours and continued at a low level on the diet poor in proteins. As soon, however, as the diet was changed back to the ordinary (rich in proteins and in some amino acids) the volume of the 24 hour urine increased at once from 38 cc. to 76 cc. and continued at a high level.

The amount of urea in the urine increased steadily with advancing age from 200 mgm. at the age of 42 days to 1100 mgm. at the age of 90 days, even when the weight of the animal was kept almost stationary by a diet qualitatively adequate but insufficient in quantity.

[1] Folin, O., and Youngburg, G. E., *J. Biol. Chem.*, 1919, **38**, 111.

Their urea excretion was approximately the same as in the control animals, although the control rabbits were over 500 gm. heavier than either one of the undernourished rabbits.

In the case of Rabbit L, the increase in the amount of excreted urea nitrogen was not nearly so pronounced as in the other rabbits, which may have been due to the fact that this animal did not receive an adequate amount of fresh vegetables.

The case of Rabbit R is interesting because the animal died of starvation. A few days before death, when the weight of this rabbit began to decline, the amount of urea nitrogen excreted in the urine increased considerably, from 58 mgm. to 510 mgm. This is fully discussed by Morgulis[2] under *"Premortal Rise in Nitrogen Elimination."*

The amount of daily urea nitrogen seems to agree fairly well with the findings of Christman and Lewis,[3] and Addis and Watanabe,[4] in adult rabbits. The work of Christman and Lewis also shows that the addition of certain amino acids causes a definite increase in the urea nitrogen of the urine of rabbits, which is in agreement with the results of my experiments.

The absence of urine in the bladder of fetal and of new born rabbits appears to indicate that urea excretion does not begin until after the birth of the animal. Some stimulus appears to be responsible for the inception of the kidney activity. The nature of this stimulus has not been discovered.

Conclusions. 1. It is definitely shown that the urea excretion in the rabbits' urine is very low at birth and gradually increases with advancing age of the animal. 2. The amount of urea in the urine increases with the age of the animal, even when its body weight is kept stationary by a qualitatively adequate diet when fed in insufficient amount. The effect of change in body weight on the urea excretion is of secondary importance. 3. The work of Christman and Lewis[3] as shown in their Tables 1 to 4 is confirmed by these experiments; that the urea in the rabbits' urine increases on diets rich in proteins and decreases on diets poor in proteins and in certain amino acids.

My thanks are due to Professor I. E. Wallin for his interest and suggestions.

[2] Morgulis, S., "Fasting and Undernutrition," E. P. Dutton & Co., N. Y., 1923, 135.

[3] Christman, A. A., and Lewis, H. B., *J. Biol. Chem.*, 1923, **57**, 379.

[4] Addis, T., and Watanabe, C. K., *J. Biol. Chem.*, 1917, **28**, 251.

5148

The Resolution of Inactive Cystine.

VINCENT DU VIGNEAUD AND LEONORE HOLLANDER.
(Introduced by W. C. Rose.)

From the Laboratory of Physiological Chemistry, University of Illinois.

Ever since the earlier investigators found that in the preparation of cystine continued hydrolysis with acid decreased the rotation of the cystine and resulted in the disappearance of the typical hexagonal crystals of cystine and the appearance of needle-like crystals, the question as to the identity of this product has been the subject of repeated investigation. Morner[1] heated horn with hydrochloric acid for 2 weeks and obtained cystine partly in needle form which was inactive. Rothera[2] made similar observations on the length of hydrolysis. Neuberg and Mayer,[3] studying the inactive material suggested the possibility that it was either meso cystine or a racemic mixture. They grew *Aspergillus niger* on some of the inactive material and obtained a dextro-rotatory product having a rotation of +93.78° while a 1% solution of l-cystine in 1 *N* HCl acid gives a rotation of about —210 at 26°. Van Slyke[4] and Plimmer[5] both found that on boiling l-cystine with 20% HCl acid it was converted into a more soluble form yielding a more soluble phosphotungstate. Recently Gortner and Hoffman[6, 7] investigated the inactive cystine and synthesized for comparison many derivatives of the l-cystine and the inactive cystine. In all cases the derivatives had the same empirical formulas but in no instance did they find them to have the same melting point and in but few instances to be identical in crystal form. They attempted to obtain dextro cystine from the inactive material by growing *Aspergillus niger* on the inactive material but no optically active material was obtained. They tried to resolve the inactive benzoyl-cystine into its optical isomers but upon hydrolysis of the various fractions the cystine obtained was completely inactive. They suggested that the inactive cystine might be the internally compensated meso form. Andrews[8] concluded that the inactive cystine

[1] Morner, *Z. Physiol. Chem.*, 1899, **28**, 595.
[2] Rothera, *J. Physiol.*, 1905, **32**, 175.
[3] Neuberg and Mayer, *Z. Physiol. Chem.*, 1905, **44**, 472, 498.
[4] Van Slyke, *J. Biol. Chem.*, 1911, **10**, 15.
[5] Plimmer and Lowndes, *Biochem. J.*, 1927, **21**, 247.
[6] Hoffman and Gortner, *J. Am. Chem. Soc.*, 1922, **44**, 341.
[7] Gortner and Hoffman, *J. Biol. Chem.*, 1927, **62**, 433.
[8] Andrews and de Beer, *J. Phys. Chem.*, 1928, **32**, 1031.

was a mixture of the meso and racemic forms and predicted that d-cystine is 4 times as soluble as l-cystine and that the d-cystine would have a lower numerical rotation than the l-cystine.

In spite of the negative results previously obtained in the attempt to resolve inactive cystine, the question was reinvestigated by using other derivatives for the resolution. Furthermore the preparation of d-cystine was highly desirable not only in order to study its chemical and physical behavior but also its physiological behavior.

The resolution of inactive cystine was accomplished by means of the brucine salt of acetyl-cystine. Since cystine is decomposed by acetic anhydride and pyridine[9] the acetylation was attempted through the action of acetic anhydride on an alkaline solution of cystine.* The brucine salt was then prepared from the acetyl cystine. The dried brucine salt of acetyl-l-cystine melted indistinctly at 158-160° and had a rotation of $(\alpha)_{D}^{27°}$—66° in water, the concentration being one per cent. From the brucine salt l-cystine was obtained having a rotation of $(\alpha)_{D}^{25°} = -205°$.

The inactive cystine was prepared by the method of Hoffman and Gortner[6] and the brucine salt prepared as in the case of l-cystine. Upon recrystallization from water the negative rotation decreased, finally becoming positive. After 12 recrystallizations the rotation appeared to remain constant at a value of +17.5° for a one per cent solution. Upon hydrolysis of the salt d-cystine was obtained in hexagonal platelets having a rotation of $(\alpha)_{D}^{25°} = +200°$ in 1% solution in 1*N* HCl. Analyses agreed with that expected of cystine. Recrystallizations using methyl alcohol have given even better results than with water. Other salts and solvents are being tried to find the optimum conditions for effecting the separation.

The resolution of the inactive cystine with the isolation of dextro cystine has not of course ruled out the possibility of there being present a small amount of meso-cystine. It is hoped that a thorough investigation of the various fractions may throw some light on this possibility. The isolation of dextro cystine affords an opportunity for the study of the utilization of this isomer by the animal body, an investigation of which is planned in this laboratory.

[9] Dakin and West, *J. Biol. Chem.*, 1928, **78**, 91.

* This method was also tried independently by C. S. Marvel and A. White with successful results. Private communication.

5149

Effects on Nervous System of Embryonic Transplantation of Spinal Cord in the Anuran, Discoglossus pictus, Otth.

RAOUL M. MAY.

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A piece of embryonic spinal cord, measuring 1 mm. to 1.5 mm, was transplanted, together with the surrounding tissues, in embryos of *Discoglossus pictus,* Otth., at the tail-bud stage. The transplantations were homoioplastic, and were made in the region of the hind limb. A histological study was made at or after metamorphosis.

Three forms of development took place, conditioned by the development of the tissues: (a) The grafted spinal cord developed independently of the homolateral hind limb, and these 2 organs had no mutual connections. (b) The transplanted tissues inhibited, through their own growth, the development of the homolateral hind limb. (c) Nervous connections occurred between the implanted spinal cord and the hind limb on the same side.

(a) The grafted spinal cord had a tendency to acquire a structure closely resembling that which it would normally have had. Motor-horn cells were irregularly distributed throughout, but there existed no distinct motor-horns. It gave rise to nerves which innervated the surrounding tissues. In one case where a nasal rudiment had been grafted anteriorly to the implanted spinal cord the latter sent forward a nerve which became fused with the olfactory organ.

(b) When the implanted spinal cord developed independently of the hind limb on the same side, the autochthonous spinal cord and its ganglia had a symmetrical structure. But in certain cases the implantation of the embryonic spinal cord inhibited completely the development of the homolateral hind leg. In such cases the lumbo-sacral plexus was not present on the affected side. The autochthonous spinal cord and its ganglia were asymmetric and had suffered a marked reduction on the same side, especially at the level of the segments which normally give rise to the lumbo-sacral plexus. The reduction was particularly marked in the ganglia, but it also occurred in the white matter of the autochthonous spinal cord and, to a less degree, in its gray matter. The motor-horn was absent from the cord in its affected segments, on the side of the implantation.

(c) In 2 cases the grafted spinal cord gave rise to a large, well formed nerve, which usurped the functions of the normal lumbo-

sacral plexus and innervated the homolateral hind leg. In these cases there existed no lumbo-sacral plexus on the side of the implantation, and the autochthonous spinal cord and its ganglia were asymmetric and reduced on that side. The reduction concerned the ganglia, and the white and gray matter of the autochthonous spinal cord, especially at the level of the segments of the absent plexus. The motor-horn here also was lacking in the autochthonous spinal cord on the affected side. The reduction of the motor cells was both ponderable and numerical.

These results show that the absence of innervation to a hind limb, due to the latter's absence or to its innervation by a supernumerary spinal cord, reverberates on the autochthonous spinal cord and its ganglia through a sensory and motor hypoplasia. The ganglia are very much reduced on the side, and along the segments, from which the lumbo-sacral plexus is absent; the lateral half of the spinal cord also undergoes a marked reduction of its white and gray matter on that side and along those segments. This latter reduction is easily observable through the absence of the motor-horn, and by a marked asymmetry. There is, besides, and up to a certain degree, a repercussion of the reduction in the neighboring segments. It therefore follows that the hind limb innervation plays a part in the development of both the sensory and motor neurones of the neuraxis.

5150

The Water and the Phosphorus Combinations of Degenerating Nerves.

RAOUL M. MAY.

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I have shown,[1] by means of microchemical methods, that the lesion of a cerebral hemisphere of the guinea pig gives rise to an increase in the water, nitrogen, and sulphur content, and to a decrease in the phosphorus content of the disintegrating cerebral matter. In the present research I have studied the variations of the water and phosphorus in degenerating peripheral nerves of the rabbit.

The sciatic nerves of one side were sectioned high in the thigh of 9 rabbits; they were later analyzed variously, from the 7th to the 196th day after the operation, the normal contralateral sciatic nerve serving as a control. All the analyses, which were made in dupli-

[1] May, R. M., *Bull. Soc. Chim. Biol.*, 1927, **9**, 970; 1929, **11**, 312.

cate on the individual nerves, were concerned with the water, the total phosphorus, and the following phosphorus combinations: lipidic, alcohol-soluble, proteidic, and water-soluble. Simultaneous histological preparations were made in all cases.

The water was determined separately after desiccation at 90° C. to constant weight. The phosphorus combinations were first extracted by means of absolute alcohol and ether, later by boiling water, in a Kumagawa micro-apparatus. After the first extraction the alcohol and ether were gotten rid of, and there were successively separated from the residue: (1) the lipidic fraction, by means of a mixture of ether and benzene, (2) a first water-soluble fraction, (3) the alcohol-soluble fraction. The first water-soluble fraction was later added to the product of the aqueous extraction. The remaining proteidic fraction was analyzed separately.

The various fractions were dried and then decomposed by sulphuric and nitric acids; the phosphorus content was determined in each of them by the previously described[1] microchemical method.

The water increased after the lesion, during the first month, at a rate which varied near 14% above its normal value in the unoperated nerve; later it decreased to a value which approached that in the control nerve (generally 64 to 67%).

The total phosphorus, established through a direct analysis, and through an addition of the phosphorus percentages in the 4 fractions, gave results which differed among themselves only within the limits of probable error. The total phosphorus decreased progressively during the first 2 months of degeneration, reaching a third of its normal value, which varies in different nerves from 1.01 to 1.24% of the dry nerve matter.

The most marked decrease was that of the lipidic phosphorus (phosphatids), which originally constitutes 50 to 60% of the total phosphorus. This fraction fell to one-tenth of its normal value after 100 days of degeneration. At this period a histological study shows the complete resorption of the lipidic constituents of the nerve. It appears probable that lipidic phosphorus which is still present after 100 days of degeneration is that of the phosphatids which are contained in the Schwann (sheath) cells and in the phagocytes which are always present in degenerating nerves.

There exists in nerves a phosphorus fraction soluble in alcohol, but insoluble in a mixture of ether and benzene, or in water; its composition is unknown as yet. This fraction decreased to a third of its normal value, which varies from 10 to 20% of the total phosphorus, after the first month of degeneration.

The proteidic phosphorus, after a rise during the first few days following the operation, fell to a third of its normal rate, which varies from 10 to 20% of the total phosphorus.

In all cases of diminution, the lowest level, once reached, was the one where the phosphorus values remained, there being no subsequent rise during the ulterior course of the nerve degeneration.

The water-soluble phosphorus fraction was the only one which increased during the degeneration. This rise, which was greater than that of the water, went beyond the normal rate (which varies from 14 to 24% of the total phosphorus) by 25% during the first days of degeneration, increasing up to 35% six months after the operation.

To a disintegration of the complex cellular constituents (phosphatids, nucleo-proteids, etc.) there thus corresponds a marked increase of the water-soluble phosphorus compounds, which very probably constitute the end-products, as simple substances, of these complex combinations. Nervous degeneration appears to bring back to the water-soluble form, in which they entered the organism, the phosphorus compounds which took part in the building up of its master tissue. However, there is also the possibility that the increase of the water-soluble phosphorus may be the expression of an incomplete synthesis in the degenerating nerve.

On the other hand, the small variations of the water, the total phosphorus, and the phosphorus combinations, in the unoperated nerves, are purely individual, and have no connection with the degeneration of the contralateral nerves.

5151

A Quinhydrone-Collodion Electrode of Special Applicability in Experimental Pathology.

JOHN C. BUGHER. (Introduced by A. S. Warthin.)

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All the accepted methods of determining the hydrogen ion activity of biological fluids require extensive alteration and manipulation of the fluid itself. If the hydrogen electrode is used, the solution must be saturated with hydrogen; if an oxidation-reduction system is set up, as with quinhydrone, the solution must be saturated with

the substance comprising the system. The only exception to this is the glass electrode, which for several reasons is unsuited to routine work.

There appeared to be a real need for an electrode which would be precise in potential values, reasonably rapid in its responses, simple to prepare and, above all, capable of functioning without alteration of the solution being examined. Such an electrode obviously could not be a gas electrode; and of the possible oxidation-reduction systems, the utilization of quinhydrone appeared the most feasible since this system has been well studied.

In principle, the device which we have called the quinhydrone-collodion electrode consists of a very minute collodion sac, the interior of which contains solid quinhydrone. On immersing the sac in the fluid to be examined, the interior, which may have contained water at first, rapidly reaches equilibrium with the exterior with respect to the readily diffusible molecules and ions. Quinone and hydroquinone liberated by the quinhydrone diffuse through the collodion membrane very slowly so that in effect a very small portion of the fluid is isolated and saturated with the quinhydrone. A platinum or gold wire introduced into the interior of the sac will acquire a potential which will be a function of the hydrogen ion activity of the contained fluid. If, now, a reference half cell is connected by a suitable bridge to the fluid outside the sac, the potential difference measured will be the sum of the electrode potential within the sac and the membrane potential due to the unequal distribution of the ions. From considerations advanced by Donnan, it may be shown that in solutions of electrolyte concentration 0.01 M or greater, the membrane potential is negligible so that the quantity measured is the electrode potential only and of the same magnitude that would be obtained if the entire solution were saturated with quinhydrone.

To construct the electrode, a short piece of platinum wire of any desired size is sealed in the end of a glass tube 3-4 mm. in diameter and 10-20 cm. in length. A small amount of mercury is placed in the interior to act as a bridge between the platinum wire and a small copper wire which serves as a conductor to the outside. The platinum wire is allowed to project 3-4 mm. beyond the seal externally and is given a thin plating of gold. It is then heated sufficiently to fuse the gold and produce a smooth gold surface. By repeatedly dipping the gold surfaced wire into a saturated solution of quinhydrone in ether, a deposit of small crystals of quinhydrone is established. Without allowing the ether to evaporate entirely, the electrode is dipped into a 4 to 6% collodion and while held horizon-

tally is rotated until it is dry. A second dipping and drying is advisable.

The electrode vessel may be of any type; but in general a flow past the electrode is desirable. A very useful form is one in which is incorporated a thermocouple as a means of measuring the temperature at the electrode.

The equations of state derived from theoretical considerations may be verified experimentally. The preliminary variations of the electromotive force are due to diffusion only and equilibrium is reached in approximately 3 minutes, the actual time depending upon the thickness of the membrane and the amount of quinhydrone deposited. By equilibrium is meant a value within one millivolt of the true equilibrium value.

The diffusion rates of quinone and hydroquinone through the collodion membrane are practically identical so that there is a gradual loss of the solid quinhydrone without important disturbance of the quinone-hydroquinone ratio.

The electrode is stable in such fluids as whole blood where the usual electrometric methods fail, the membrane protecting the electrode system. Excellent results may be secured with the electrode in whole blood in experiments running for one half to one hour. Where it is important to maintain a constant partial pressure of gases above the solution this electrode is convenient inasmuch as it may be incorporated in a closed system.

5152

Etiology of Acute Upper Respiratory Infection, (Common Cold).*

PERRIN H. LONG AND JAMES A. DOULL.

From the Department of Medicine and the Clinical Laboratory of the John J. Abel Fund for Research on the Common Cold, The Johns Hopkins University.

In 1914, Kruse[1] reported that he was able to reproduce the common cold in human volunteers by introducing into their nasal passages small amounts of Berkefeld filtrate of the nasal secretions from individuals suffering from natural colds. This observation

* This investigation was carried out under The John J. Abel Fund for Research on the Common Cold.

[1] Kruse, *München Med. Wchnschr.*, 1914, **61**, 1547.

has been confirmed by Foster[2] and by Olitsky and MacCartney.[3] Dochez, Shibley and Mills[4] have been able to infect apes by means of intranasal inoculations of Berkefeld V. filtrates of the nasal washings from natural human colds. However, in all positive experiments a gram negative anaerobic bacillus of the type described by Olitsky and Gates,[5] was cultivated from the Berkefeld filtrates. In a later report Shibley, Mills and Dochez[6] stated that they had been unable to infect apes by means of intranasal inoculation of filtrates of nasal washings from healthy individuals, although the majority of these filtrates contained gram negative anaerobes of the type described by Olitsky and Gates. These findings led them to believe that the type of upper respiratory tract infection under consideration was caused by filterable virus. Recently Shibley and Dochez[7] have described an upper respiratory tract infection in man, which followed the intranasal inoculation of filtrates of the nasal washings from natural human colds.

However, as has been pointed out by Olitsky and MacCartney and by Dochez and his associates, the Berkefeld V. and N. filtrates of the nasal washings from colds frequently contain minute filter-passing bacteria. Thus, while it has been demonstrated that the infectious agent of the common cold will pass the Berkefeld V. and N. filters, the question whether the filter-passing anaerobes constitute the infectious agent, or whether it is a true filterable virus, has not been definitely settled.

Recently we have attempted to transfer colds to healthy young women volunteers, by means of filtrates containing the filter-passing anaerobes and by means of bacteria-free filtrates. These volunteers were placed in strict isolation and every precaution was taken to protect them from sources of natural infection. The experiments were carried out during the months of June and July, 1930, in which period the incidence of colds in Baltimore was at a low level.

The nasopharyngeal washings were obtained during the first 48 hours of the natural colds. Half of each washing was filtered through a Berkefeld V. candle and in every instance the filtrates contained filter-passing anaerobes. The other half of the washing

[2] Foster, G. B., Jr., *J. Am. Med. Assn.*, 1916, **66**, 1180.

[3] Olitsky, P. K., and MacCartney, J. E., *J. Exp. Med.*, 1928, **38**, 691.

[4] Dochez, A. R., Shibley, G. S., and Mills, K. C., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 562.

[5] Olitsky, P. K., and Gates, F. L., *J. Exp. Med.*, 1922, **36**, 501.

[6] Shibley, G. S., Mills, K. C., and Dochez, A. R., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 59.

[7] Shibley, G. S., and Dochez, A. R., communication to the Soc. for Clin. Invest., Atlantic City, May 5, 1930 (unpublished).

was filtered either through a small improved Seitz filter (Uhlenhuth model) or through a Berkefeld W. candle, and all attempts to demonstrate aerobic or anaerobic microörganisms in these filtrates by means of improved culture methods have been without success.

After a preliminary period of isolation (to preclude the chance of a natural infection arising), the subjects were inoculated intranasally and the pharynx was swabbed with small amounts of the same filtrate. In the first group of experiments, 10 volunteers were inoculated, half of them receiving Berkefeld V. filtrates and the other half Seitz filtrates. Two subjects from each group developed colds. From one of those developing a cold subsequent to the inoculation of Berkefeld V. filtrates, serial passages were made through 2 subjects by means of Seitz filtrates. In a second experiment successful serial transmissions were accomplished through 3 individuals by means of Seitz filtrates and the infection was carried through a fourth passage by means of Berkefeld W. filtrates. All volunteers were examined, both before and after inoculation, by independent observers.

To recapitulate: Of 5 individuals inoculated with Berkefeld V. filtrates of the nasopharyngeal washings from natural colds, 2 developed colds. Nine of 15 individuals inoculated with Seitz or Berkefeld W. filtrates of nasopharyngeal washings from acute colds, developed colds. Serial transfers through 2 and 4 individuals respectively, have been accomplished by means of Seitz or Berkefeld W. filtrates. All of the Berkefeld V. filtrates contained filter-passing anaerobes, while in all instances both aerobic and anaerobic cultures from the Seitz and Berkefeld W. filtrates remained sterile. These findings confirm and extend the observations of Kruse, Foster and of Dochez and his associates, that the infectious agent of acute upper respiratory infection (the common cold) is a filterable virus.

5153

Stillbirths in a Mouse Interspecific Cross (Mus musculus x Mus bactrianus)

C. V. GREEN. (Introduced by C. C. Little.)

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Reports of species crosses among rodents have been infrequent in the literature and in most of these only small numbers were involved.

A general phenomenon of such crosses has been the presence of heterosis. This has been manifested under several forms such as large size of the hybrids, longevity and increased fecundity. Another criterion might be the frequency of stillborn young since, as Feldman[1] concluded in the case of rats, stillbirths are due at least partly to impaired vigor of the female. Thus the paucity of stillbirths among the offspring of hybrid females would be a relative measure of augmented vigor. The present note presents some data on this point.

The forms used in this cross were the Little inbred strain of dilute brown non-agouti *Mus musculus* and a stock of *Mus bactrianus,* the original animals of which were captured near Peiping, China, in 1926. They are small, light-colored, white-bellied murids (genetically, intense black agoutis) intermediate between the subspecies *gansuensis* Satunin and *tantillus* Allen,[2] on the whole nearer the former. They mate readily with *musculus* and the offspring of both sexes are fertile. The first generation hybrids are intermediate in body weight although in such skeletal measurements as skull length, femur length and tibia length they usually equal or exceed the larger parent. Heterosis is markedly manifested in the increased litter size of the females, their litters averaging 6.4 ± 0.12 as opposed to 5.4 ± 0.04 for the straight dilute brown non-agouti *musculus* and 4.6 ± 0.11 for *bactrianus.*

Perhaps the most striking characteristic of increased vigor of the hybrid females, however, is the extremely rare occurrence of stillbirths among their offspring. The following table summarizes the data for the several types of matings.

TABLE I.

Mating	Living Young ♂	Living Young ♀	Stillborn Young ♂	Stillborn Young ♀	Stillborn Young ?	Total Born	Total Stillborn	% Stillborn
1. *bactrianus* ♀ x *bactrianus* ♂	228	216	6	5	2	457	13	2.84 ± 0.53
2. *musculus* ♀ x *musculus* ♂						7416	804	10.84 ± 0.24
3. *musculus* ♀ x *bactrianus* ♂ *musculus*	106	105	8	3	2	224	13	5.80 ± 1.05
4. *musculus* ♀ x *bactrianus* ♂ *musculus*	287	242	18	26	9	582	53	9.11 ± 0.80
5. *bactrianus* ♀ x *musculus* ♂	307	258	3	2		570	5	$.88 \pm 0.26$

The percentage of stillborn in the inbred *musculus* stock (2) is rather high (10.84 ± 0.24) as compared with the 1.3% which King[3]

[1] Feldman, H. W., *Carnegie Inst. Wash. Pub.*, 1926, 337, 49.

[2] Allen, G. M., *Am. Mus. Novitates,* 1927, 270.

[3] King, H. D., *Anat. Rec.*, 1921, **20**, 321.

found in her rats. The *bactrianus* mice (1) show a lower proportion (2.84 ± 0.53%) while the percentage of the hybrids (3) which were born dead is intermediate. This may be the result of small numbers or it may be an indication of the innate vigor of the F_1 fetuses, since Little[4] found significantly fewer stillbirths resulting from human interracial than from intraracial matings. The most illuminating figures are those for the 2 types of back-cross matings (4 and 5 in the table). These two types have the same genetic constitution so the great difference in the percentage of stillbirths (8.23 ± 0.84) must be due to the mother and exhibits the remarkable degree to which heterosis is present in the female hybrid.

5154

Oxydative Blood Destruction and Blood Pigments Protection.

KONRAD BINGOLD. (Introduced by E. F. Müller.)

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Contrary to the generally accepted idea that destruction of blood pigments occurs gradually it could be demonstrated by newer experiments that any oxydate destruction up to colorless end products may occur directly with the oxyhemoglobin (hematin acid, succinic acid) and that intermediary products (methemoglobin, hematin, porphyrin) do not appear.

This result can be proved: (1) by bacteriological examinations: Using agar prepared with boiled blood, it can be found that the destruction of blood pigments occurs with formation of peroxyd by certain bacteria and with decolorization of hematin. (2) by spectroscopical examinations. Blood containing hematin loses its color instantaneously if combined with H_2O_2. No intermediary products can be found with spectroscopical examination.

For the assumption of an oxydative destruction two different factors have to be taken into consideration. (1) A protective factor has to be assumed. This must be a product that is close to catalase. According to our last studies very small amounts of fresh blood are sufficient to protect the blood pigments (hemoglobin, methemoglobin, hematin) from an oxydation by H_2O_2. This protective action can be increased when different media are used. The

[4] Little, C. C., PROC. SOC. EXP. BIOL. AND MED., 1919, **16**, 127.

smallest effect will be found by a dilution of water, normosal acts more effectively and still more intensive is the effect in urine; serum and bile show the most intensive action. The pH is here of great importance.

The oxydative action of H_2O_2 can be increased hypernormally if no blood-pigment protection is present. Proof has been found in the following experiment: A dilute methylenblue solution will not be altered by H_2O_2; it remains blue. If one adds "unprotected" (free from catalase) hgb. or Ht. crystals it is seen that not only blood pigment solution loses its color but also the methylenblue solution becomes water clear. The destruction of blood pigments therefore occurs only under the influence of oxydation. Peroxyd (H_2O_2) destroys very rapidly the blood pigments if the protective ferment has been destroyed.

This occurs as demonstrated: (1) Under bacterial influences (staphylococci, f. i. destroyed in blood agar cultures). (2) Under the influence of high temperature. The human "protective substance of blood pigments" will be destroyed if a temperature of 73°C. is reached, and furthermore a decoloration becomes evident under the influence of H_2O_2. With animals the destructive temperature differs from that in men. Dog's blood for instance will be destroyed at 50° C. while pigeon blood is destroyed at normal temperature. Similar to H_2O_2 is the effect of peroxyd-like influence of living bacteria. This can be easily demonstrated if pneumococci are cultivated on agar that has been mixed with boiled blood. According to Wieland it has to be assumed that O_2 in the metabolism will be changed intermediary into hydroperoxyd and that the catalases have the function of almost instantaneously destroying the H_2O_2. It is possible that the blood-pigment protective body has under such conditions been destroyed previously.

Observation with blood containing urine in cases of gas bacilli septicemia leads to the opinion that a loss of the blood-pigment protective body may, under certain conditions, take place in the body itself.

5155

The Cause of Death in Experimental Acute Diffuse Peritonitis.*

BERNHARD STEINBERG.

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There are several current conceptions regarding the cause of death in acute diffuse peritonitis. Askanazy[1] considers the intestinal paralysis with dilatation of lymphatics and pressure upon the ganglion cells responsible for death. Lennander[2] agrees with the intestinal paralysis view but holds that the passage of toxins and bacteria through the paralyzed intestinal wall is the causative factor in fatal terminations. Whatever factor the intestinal obstruction plays in the fatal outcome (Orr and Haden[3] do not believe that sufficient evidence has been presented to justify the belief that intestinal obstruction is alone the cause of death) it is of secondary importance. The intestinal paralysis constitutes one of the several complications incident to an acute diffuse peritonitis. Steinberg and Ecker[4] presented evidence in experimental colon bacillus peritonitis that the soluble toxic substance of the bacillus produces death and possibly the coincident complications. Additional evidence that the bacterial toxin is the primary death producing factor has been furnished by Williams,[5] who demonstrated that *B. welchii* toxin is responsible for death in acute diffuse peritonitis from obstruction of the small intestine.

The experiments here reported have been undertaken to obtain further facts of the rôle of the bacterial toxin in acute peritonitis. Diphtheria bacillus was used because it is a definite toxin producer and the antitoxin is readily available. Diffuse peritonitis was pro-

* This work is a part of a general investigation on "Recovery in Peritonitis" made possible by a grant by the Committee on Scientific Research of the American Medical Association.

1 Quoted by Heineke, H., *Deutsches Arch. f. klin. Med.*, 1900-1901, **49**, 429.

2 Quoted in Kaufmann's Pathology by Reimann, **2**, 865.

3 Orr, T. G., and Haden, R. L., *Arch. Surg.*, 1929, **18**, 2159.

4 Steinberg, B., and Ecker, E. E., *J. Exp. Med.*, 1926, **43**, 443.

5 Williams, B. W., *Brit. J. Surg.*, 1926, **14**, 295.

duced by the intraperitoneal introduction into dogs of diphtheria bacilli suspended in a 2½% suspension of gum tragacanth in saline. It has been demonstrated[6] that bacteria suspended in gum tragacanth invariably produce a fatal peritonitis.

Nineteen dogs were injected. Each animal received intraperitoneally the washings of 3 slants of a 24-hour diphtheria bacillus culture suspended in 40 cc. of a 2½% suspension of gum tragacanth. The injection was made with syringe and needle through the abdominal wall. Ten of these 19 dogs were given 20,000 units of diphtheria antitoxin each by routes indicated on the chart. The antitoxin was given a few minutes after the production of peritonitis. The 9 dogs *without* antitoxin died in within 18 to 48 hours. All of the 9 dogs had a marked fibrino-hemorrhagic peritonitis. The 10 dogs that received diphtheria antitoxin survived. They were slightly ill during the first 18 hours but after that they were apparently well. One of the 10 dogs that survived was killed 56 hours after production of peritonitis. The animal had a moderate fibrinopurulent peritonitis. The result of further experiments with a detailed description of the differences in the type of peritonitis in the passively immunized and in the non-immunized dogs will be reported elsewhere at a later date.

TABLE I.

3 slants of diphtheria bacilli in 2½% gum tragacanth injected intraperitoneally.

No. of Dogs	Diphtheria Antitoxin Given	Route of Antitoxin Administered	Outcome
9	None	————	Died 18 to 48 hours with fibrino hemorrhagic peritonitis.
6	20,000 units	Intravenously	Survived
2	20,000 "	Intramuscularly	"
1	20,000 "	Intraperitoneally	"
1	20,000 "	Subcutaneously	"

Summary: Further evidence is submitted that death in acute diffuse peritonitis under the conditions of these experiments is due to passage of a bacterial toxin from the peritoneal cavity. The toxin is elaborated by the introduced bacteria within the peritoneal cavity. Death can be prevented by the administration of the corresponding antitoxin. The local inflammatory reaction *per se,* apparently, has no effect on the survival or death of the animal.

[6] Steinberg, B., and Goldblatt, H., *Arch. Int. Med.*, 1927, **39**, 446.

5156

A Technique for Recording Electrical Changes in Isolated Chick Embryo Hearts.

HERBERT POLLACK. (Introduced by Shields Warren.)

From the Laboratory of Pathology, Palmer Memorial Hospital, Boston, Mass.

It was thought that a technique of recording the electrical changes in the isolated warm blooded heart would offer possibilities of determining the direct effect of many drugs. The plan of this work is to study (a) the functional changes as recorded by the electromyograms; (b) chemical changes by subsequent analyses; (c) histopathological changes.

Towards this end the following technique was devised and has proved to work satisfactorily in preliminary experiments. Fertile chicken eggs are incubated to the desired stage. The embryos are removed from the shell and pinned out on a paraffin block. The heart is dissected out carefully, leaving a fairly long stalk of great vessels and some tissue. This avoids injury to the auricles and allows for handling. The isolated heart is then placed in a receptacle.

The receptacle used is a small glass dish cemented to an ordinary microscopic slide, which is set on a mechanical stage. The dish is filled about half way with paraffin, to allow for fixing the heart in place.

The electrodes consist of 2 zinc-zinc sulphate cells of simple construction. An ordinary test tube is drawn to a capillary about 3 inches from its mouth. The constricted portion between the capillary tip and body of the tube is bent out at about a 60° angle. The capillary tip is then bent down in the complementary angle at a point where it extends beyond the outer wall of the tube, and cut to leave a tip of a few millimeters. This is done to allow approximation of the two electrodes. A glass slide is cemented to the body of the tube with DeKhotensky for fixation to mechanical stages, by means of which motion is obtained in 3 dimensions. The capillary tip is threaded with several thicknesses of cotton string. These should be snugly packed in the capillary opening. Too tight a fit interferes with capillarity and causes poor wetting, which tends to increase the resistance. The thread should be run well up into the body of the tube. The outside string is cut about 0.5 cm. from the opening and wound with a circular thread. Sodium chloride solution (0.9%) is used to saturate the string, which serves as the immediate contact with the heart. This tube is filled with a thin paste of kaolin and

saturated zinc sulphate. The zinc poles are pushed as far down into the tube as possible. The electrodes have a resistance varying from 2000 to 4000 ohms. They can be used for constant recording over periods of several hours without showing an appreciable amount of polarization or resistance changes.

In order to maintain the hearts at proper temperature the electrodes are set up inside an incubator.

Attempts were made to use as medium Ringer's, Tyrode's, Tyrode's without glucose, Locke's, and Locke's without glucose. For reasons which will be discussed at a later date these were discarded in favor of the bare heart. Proper moisture conditions could be maintained by saturating the air of the incubator with water vapor. A shallow enamel pan of the type used in photography is placed on the bottom of the incubator. This is filled with water. An immersion "Hot Point" heater is set in the water and the cord run out through a ventilating hole in the wall of the incubator.

Alternating currents are one of the most disturbing factors encountered in the development of the technique. These can be reduced to a minimum by proper shielding. If an electric incubator is used all wires must be entirely disconnected by removing the plugs from the sockets during the time when the string galvanometer is in the circuit. The cooling of the incubator when the heating currents are off is minimized if the pan of water has been brought to a boil before disconnecting the current.

Three leads from the heart are feasible in embryos over 4 days. The attempt was made to keep them referable to the usual clinical leads. Lead α is taken across the two auricles, lead β is taken with one electrode on the right auricle and the other at the apex, γ is left auricle and apex. The records of various stages are consistent when taken at different times. Minor variations in the position of the apical electrodes in lead β and γ do not affect the fundamental picture.

5157

Developmental Aspects of the Electrocardiogram.

HERBERT POLLACK AND MAURICE DIONNE.
(Introduced by Shields Warren.)

From the Laboratory of Pathology, Palmer Memorial Hospital and the Lahey Clinic, Boston, Mass.

By means of the technique previously described[1] a series of electrocardiograms of the developing chick embryo from the fourth to the fifteenth days inclusive, were taken.

Three distinct waves are seen as early as the fourth day, which apparently represent P, R, and T waves. The recorded voltages with a circuit resistance of 4500 ohms are P = .025 millivolts, R = .05 millivolts, and T = .05 millivolts at this stage of development. By the seventh day the cardiogram assumes the shape that it maintains throughout the period of incubation.

The alpha lead is always the lowest in amplitude. The P and R waves are upright. Frequently the T wave originates directly from the R and is upright or diphasic.

The beta lead shows upright P and R waves. The S wave may be present and varies from 0.3 to 1.0 millivolt. The T wave is usually the wave of greatest amplitude, going up to 2 millivolts in the 14-day hearts. It is usually inverted, resembling a fish hook in shape. When the S wave is present the T takes its origin directly from it before the isopotential has been reached.

The gamma lead resembles the beta very closely, though its amplitude is lower.

The rates vary from 50 to 120, generally in the neighborhood of 60. For the individual heart the rate is constant. Arrhythmias are frequently found, the commonest type being the sino-auricular block. Coupled beats are not infrequent. Ventricular extra systoles are rare. Nodal rhythm has been found with the P wave occurring in the crest of the T.

Complete correlation of the developmental morphology with the electrocardiograms depends on recording tracings before the fourth day of incubation, as the 4 chambers and septa are present by this time. The finding of 3 waves on the fourth day correlates with this fact.

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[1] Pollack, H., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 61.

5158

Is the Effect of Thyroid and Thyroxin upon the Metabolic Rate Specific for Vertebrates?

WM. A. HIESTAND. (Introduced by M. F. Guyer.)

From the Zoological Laboratory, University of Wisconsin.

The author, while conducting experiments on insect metabolism, measured the metabolic rate of two species of insects under the influence of thyroid and thyroxin feeding. The forms used were the wasp, *Polistes pallipes,* and the roach, *Periplaneta australasiae.* Armour's desiccated thyroid was fed mixed with honey. Squibb's thyroxin crystals were also given in a honey mixture. Carbon dioxide output was measured per hour before and after feeding. In most cases of thyroid feeding results were not pronounced enough to be considered of consequence. In one case in which one wasp fed for approximately 4 minutes (a comparatively long time) the basal metabolic rate increased to more than twice normal for the first 2 hours following feeding. After the 4th hour the rate had again returned to normal. The very slight increases and decreases obtained with feeding desiccated thyroid to roaches were not consequential. It was assumed that proportionately a very great amount of desiccated thyroid gland was necessary to produce an increase in metabolism in these insects.

Thyroxin feeding, however, produced strongly positive results with the wasps. Each time it was fed and with each individual a very definite increase in the basal rate occurred. In one instance the rate was increased 6.5 times normal for the first hour after feeding, with a return to normal at the end of the third hour. Other records of an increase of 3 to 5 times the normal rate were not rare. With roaches the results were not so striking. In many cases no effects were noted but usually a slight rise in the rate occurred, which soon returned to normal. In no case was the increase as great as 1.5 times the normal rate. Possibly the wasps being more highly organized animals were more susceptible. Also proportionately more thyroxin was consumed by the wasps whose normal metabolic rate is much higher than that of the roach.

Adequate controls were run to make sure that the increase in rate was due to the glandular extract and not simply to nutritional properties. Thus it has been shown that thyroid gland and thyroxin will increase the basal metabolism of organisms other than those having thyroid glands. The effect, however, is of decidedly short

duration. It would be interesting to determine the threshold of dosage for an active form as *Polistes*. The above animals were kept in a dark constant temperature cage with uniform conditions of moisture, so that only the basal rate was obtained. In all probability the loss in weight in other forms after prolonged feeding of thyroid found by previous workers[1] was due to an increase in oxidation and consequently a decreased fat deposition in the larval state.

It should also be mentioned here that feeding Lugol's solution (5% I + 10% KI) did not produce any marked change in the rate.

Five wasps and two roaches were used in the thyroid-feeding experiments. Twenty-five normal readings were taken with the wasps and seven experimental ones. Four normals and two experimentals were taken with the roaches. The same insects were used as checks before and after thyroid feeding. Checks were also made by feeding honey syrup alone with no thyroid or thyroxin present.

Three wasps and four roaches were used in the thyroxin experiments. Nine normal tests and three experimental ones were made with the wasps; nine normals and three experimentals also with the roaches.

5159

Toxin-Antitoxin Reactions on the Surface of Collodion Particles.

JULES FREUND.

From the Henry Phipps Institute, University of Pennsylvania.

Because of its theoretical and practical significance the mechanism of the neutralization of toxins by antitoxins is one of the most extensively investigated problems of immunology. According to Arrhenius and Madsen the toxin-antitoxin neutralization may be a chemical reaction governed by the law of mass action. On the other hand Bordet thinks that it does not follow the quantitative laws of simple chemical reactions but is an adsorption phenomenon controlled by the electrical charge of the toxins and antitoxins.[1]

[1] Kopec, S., *Biol. Bull.*, 1926, **50**, 339. Kunkel, B. W., *J. Exp. Z.*, 1918, **26**, 255. Terao, A., and Wakamori, N., *Japan Med. World*, 1924, **4**, 68.

[1] Wells, H. G., "The chemical aspect of immunity." Second edition. 1929. Bayne-Jones, S., chapter in "The Newer Knowledge of Bacteriology and Immunology" by Jordan, E. V., and Falk, I. S., 1928. Eisler, M., chapter in *Handb. path. Mikroorganismen* IV. 2 by Kolle, W., Kraus, R., and Uhlenhut, P., 1928.

It seems probable that the recent progress in the study of physico-chemical aspects of the antigen-antibody reactions[2] particularly those of agglutination, precipitation and phagocytosis is due to the circumstance that these reactions can be studied on the surface of particulate matter. Similarly it seems desirable to investigate the phenomenon of the neutralization of toxins by antitoxins on the surface of simple particles.

Loeb and his associates, Northrop, Hitchcock and Kunitz,[3] have shown that the adsorption of proteins by collodion particles makes possible the use of certain physico-chemical methods that can not be applied to proteins in solution. The possibility of studying immunological reactions on the surface of collodion particles occurred to me in connection with a study of physico-chemical aspects of the agglutination of tubercle bacillus.[4] In that work it was observed that collodion particles are acid-fast when stained by the Ziehl-Neelsen method and that they remain acid-fast even if they are coated with protein. The method of demonstrating the presence of protein film on the collodion particles by agglutinating the coated collodion particles with anti-egg-white precipitins, was not described in the paper. Jones[5] showed that collodion particles coated with various proteins are agglutinated by specific precipitins, and similar experiments were described by Hulshoff Pol,[6] who employed zinc stearat and colophonium instead of collodion particles. Bedson[7] successfully employed the method of adsorption to collodion particles in his studies on neutralization of vaccinia virus. Recently Mudd, Lucke, McCutcheon and Strumia[8] have reported that collodion particles treated with precipitogen are phagocytosed in a manner that is essentially identical with the phagocytosis of bacteria treated with bacteriotropins.

Thus far I have studied the reactions between diphtheria and tetanus toxins and antitoxins* by this method and the results can be summarized as follows:

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[2] Northrop, J. H., chapter in "The Newer Knowledge of Bacteriology and Immunology," by Jordan, E. V., and Falk, I. S., 1928.

[3] Loeb, J., "Proteins and the Theory of Collodial Behavior," 1922.

[4] Freund, J., *Am. Rev. Tuberc.*, 1925, **12**, 124.

[5] Jones, F. S., *J. Exp. Med.*, 1927, **46**, 303, 1928, **48**, 183.

[6] Hulshoff Pol, J. P. G., *Z. f. Immunitatsf.*, 1929, **61**, 99.

[7] Bedson, S. P., *Brit. J. Exp. Path.*, 1929, **10**, 364.

[8] Mudd, S., Lucke, B., McCutcheon, M., and Strumia, M., *J. Exp. Med.*, 1929, **52**, 299, 313.

* The toxin and antitoxin preparations were obtained through the courtesy of Dr. F. M. Huntoon and Dr. T. S. Githens of the H. K. Mulford Co., Philadelphia.

I. Reaction Between Diphtheria Toxin and Antitoxin. Collodion particles treated with diphtheria toxin were flocculated by diphtheria antitoxic horse-serum. When the collodion particles were treated with toxin that had been heated for 2 hours at 55° C., the flocculation by antitoxin was reduced considerably. Collodion particles treated with formalin-toxoid were also flocculated by antitoxin and previous heating of the toxoid did not affect the flocculation phenomenon.

The following is a brief description of a typical flocculation experiment: To 10 cc. of diphtheria toxin No. 296 (400 M.L.D., 0.15 L + Dose) one cubic centimeter of a heavy suspension of collodion particles was added. The mixture was gently shaken for 5 minutes and kept over night in a refrigerator. Then the collodion particles were washed 3 times by centrifugalization and suspended in 3 cc. of saline. Three drops of the suspension of treated collodion particles were mixed with one cubic centimeter of dilution of antitoxic horse-serum (not concentrated, approximately 500 units), the mixture kept in water bath of 37° C. for 2 hours and then over night in a refrigerator. The reaction was read while the tubes were gently shaken.

TABLE I.

Mixture	Dilution of Serum					
	1:10	1:20	1:40	1:80	1:160	1:320
Collodion particles treated with toxin + antitoxin	Large flocculi; clear fluid	Large flocculi; clear fluid	Large flocculi; clear fluid	Large and medium flocculi; clear fluid	Medium flocculi; clear fluid	Negative
Collodion particles treated with heated toxin + antitoxin	Large flocculi; fairly clear fluid	Large flocculi; turbid fluid	Very few large flocculi; turbid fluid	Negative	Negative	"
Collodion particles treated with toxin + normal horse serum	Negative	Negative	Negative	"	"	"

Collodion particles treated with diphtheria toxin injected into the skin of guinea pigs produces redness, edema and superficial necrosis of the skin. The inflammation was prevented when antitoxin was injected simultaneously.

In interpreting the flocculation reactions the following possibilities may be taken into consideration. The reaction may be due to the interaction (1) between the toxin and antitoxin, and essentially similar to Ramon's flocculation reaction or Hoehn's and Tschertkow's[9] precipitation reaction, or (2) between bacterial agglutinogen and agglutinins, or (3) between other antigens and antibodies (lipoid antigen[10]). The following evidence seems to support the first possibility. (a) The collodion particles are coated with a film of toxin, for they have a toxic action that can be neutralized by antitoxin; (b) heating of the toxin reduced the flocculation materially; (c) in this experiment the toxin can be substituted by formalin-toxoid; (d) heating of the toxoid has little if any influence upon the flocculation of the particles treated with toxoid; (e) the antitoxic horse serums did not agglutinate a suspension of diphtheria bacilli; (f) serum obtained from rabbits immunized with washed and heated diphtheria bacilli did not cause flocculation; (g) the flocculating capacity of antitoxic serums was not changed by contact (absorption) with diphtheria bacilli. However it should be mentioned that serums of 2 rabbits treated with formalin-toxoid did not bring about flocculation.

II. Neutralization of Tetanus Toxin Adsorbed Upon Collodion Particles by Antitoxin. Collodion particles treated with tetanus toxin were not flocculated by antitoxin. Indeed it was observed repeatedly that such particles could be more readily suspended in various dilutions of antitoxin than in saline. However, the presence of tetanus toxin upon the treated collodion particles and the neutralizing action of antitoxin was very clearly demonstrated by animal experiment. To 10 cc. of tetanus toxin (M.L.D. 0.0001 cc.) 2 cc. of a heavy suspension of collodion particles were added and the suspension gently shaken for 5 minutes at room temperature. The particles were then separated by centrifugalization and after they had been washed in saline they were resuspended in 1 cc. of saline. One tenth of a cubic centimeter of the suspension was mixed with 1 cc. of antitoxin dilution, gently shaken for 5 minutes and centrifugalized. After the supernatant fluid had been discarded, the particles were carefully suspended in 4 cc. of saline, spun again and suspended in 0.4 cc. of salt solution. Two-tenths of a cubic centimeter of the suspension was injected into the right hind leg of mice.

[9] Hoehn, E., and Tschertkow, L., *Z. f. Immunitatsf.*, 1929, **62**, 201.

[10] Bouquet, A., and Negre, L., *Ann. Inst. Pasteur*, 1923, **37**, 787. Freund, J., *J. Immun.*, 1927, **13**, 161. Witebsky, E., and Krah, E., *Z. f. Immunitatsf.*, 1930, **66**, 78. Krah, E., and Witebsky, E., *Ibid.*, 1930, **66**, 59.

TABLE II.

Days after injection	Dilution of Antitoxin							
	1:10	1:100	1:1000	1:10,000	1:50,000	1:100,000	1:1,000,000	Control
2	normal	normal	normal	normal	normal	local tetanus	local tetanus	local tetanus
4	,,	,,	,,	,,	,,	dead	dead	dead

Mice injected with the *supernatant* fluid of the control suspension, *i. e.*, collodion particles treated with toxin, remained free from tetanic symptoms; therefore the tetanus observed in these experiments was due to the toxin adsorbed by the collodion particles before and released from them after injection into the mouse.

This experiment shows clearly that tetanus toxin adsorbed upon collodion particles is neutralized in 10 minutes even by a very high dilution of antitoxin (1 in 50,000).

The question arises as to whether collodion particles treated first with antitoxic serum and then with toxin produce tetanus in mice. Collodion particles were treated (a) with antitoxic serum and then with toxin; (b) with normal horse serum and then with toxin; (c) with antitoxic serum, then with toxin and then with antitoxin again; (d) with toxin alone.

Repeated experiment showed that the order of the toxicity of particles was as follows: a>d>b>c. In other words the antitoxin probably adsorbed on collodion particles (there is no direct evidence for the adsorption of the antitoxin on the particles) failed to neutralize the toxin subsequently adsorbed, moreover collodion particles treated first with antitoxin and then with toxin were slightly but definitely more toxic than particles treated with toxin alone. In contrast to this collodion particles treated first with normal horse serum and then with toxin were less toxic than those treated with toxin alone. Collodion particles first treated with antitoxin, then with toxin and then again with antitoxin were found to be neutralized by the second contact with antitoxin.

It is interesting to compare this observation with Jones' experiment on particles treated with precipitin-serum and then suspended in precipitinogen. Jones found that "Particles exposed to immune serum and subsequently washed fail to agglutinate in the presence of antigen although some of the protein constituents of the immune serum are fixed upon them and its antibody content diminishes."

Conclusions. (1) Collodion particles treated with diphtheria toxin or formalin-toxoid are flocculated by antitoxic horse serum. This phenomenon is shown to be very probably due to a reaction

between toxin and antitoxin and not to a combination of other antigens and antibodies. (2) Tetanus toxin adsorbed to collodion particles can be neutralized by high dilutions of antitoxin.

5160

The Experimental Production of Chronic Empyema in Dogs.

W. M. FIROR. (Introduced by Ferdinand C. Lee.)

From the Department of Surgery of the Johns Hopkins Medical School.

In 1929 we became interested in a new treatment for chronic empyema. The procedure, however, was such a radical departure from the accepted ones that it seemed desirable to test its value on experimental animals, rather than on human beings. Chronic empyema virtually never occurs spontaneously in dogs. We found a paucity of literature on this subject; namely, that chronic empyema in dogs is difficult to produce.

We first opened the pleural cavity and injected into it 2 or 3 cc. of fluid abundantly infected with a variety of organisms. To secure the persistence of this infection a bit of gauze was placed in the cavity before expanding the lungs and closing the wound. These animals all died within 48 hours. At necropsy both pleural cavities were found to be heavily infected. In some there was an accumulation of hemorrhagic, purulent fluid which partially compressed both lungs; in others there was a small amount of thick pus covering all the pleural surfaces. A similar result was obtained when cultures of a single pathogenic organism were used in place of those with several types of organisms. It seemed apparent, then, that a possible explanation of the non-occurrence of chronic empyema in dogs is found in the inability of this species to localize acute pyogenic infections within the pleural cavity. The mediastinum in these animals is a thin, filmy structure, and organisms pass with ease and rapidity from one pleural cavity to the other. The pericardial sac is totally different in this respect; in all of our animals this tissue resisted the penetration of the infecting organisms. Another possible explanation for the failure of empyema to occur spontaneously in dogs lies in the fact that these animals seldom have lobar pneumonia.

The next experiments were devised with the idea of securing adhesions between the visceral and parietal pleurae previous to the

implantation of infected material. It was thought that by this procedure one might prevent the dissemination of the infection. A ring of sterile gauze, measuring about 10 cm. in diameter and 1 cm. in width, was spread over the surface of a lung, held in place simply by keeping the lungs expanded during the closure of the thoracotomy wound. In a small percentage of the cases the gauze did not maintain its position, but usually within a week firm adhesions formed around the ring and between the visceral and parietal pleurae. After a month the gauze was not only completely covered with fibrous tissue, but was thoroughly incorporated in it. On a few occasions sterile gauze that had been saturated with bismuth subnitrate was used, with the idea that the tissue reaction might be more intense and the ring would be more readily outlined on x-ray plates. However, the bismuth evoked an outpouring of fluid, which caused a bilateral hydrothorax with sufficient respiratory hindrance to cause death. After varying periods of time, usually 2 weeks, a small opening was made in the center of the thoracotomy wound. By this time the adhesions surrounding the sterile gauze ring prevented collapse of the lung and formed a well walled-off intrapleural pocket, in which cultures of virulent pneumococci were placed. These animals were watched with great care, and at no time were there any evidences of the bacteria having gained a foothold. When, however, a bit of unabsorbable foreign body was added to the bacterial culture, various types of infection resulted. In some cases an abscess would form, which would rupture spontaneously through the scar of the incision, drain for several days, and then heal. In others the infection would become walled off and apparently remain dormant for weeks, without ever communicating with either a bronchus or the outside. In still others the bacteria would spread by way of the interlobar fissure and cause an extensive bilateral pyothorax with death.

None of these results seemed to simulate empyema accurately. Accordingly, a group of animals were prepared in whom sterile gauze rings were carefully placed over the convex surface of the left lung, and in addition strips of gauze were inserted between the lobes. Three to 4 weeks later 3 cc. of a pure culture of pneumococci (Types I and III) were poured into the center of each of these artificially formed pleural pockets. Two days later the wounds were again opened and bits of gauze contaminated with a variety of organisms were placed in the same pockets. The wounds were closed with 2 or 3 deep sutures of braided silk, and with interrupted silk sutures in the skin. Within a few days these wounds formed subcutaneous abscesses

which opened and discharged pus. This purulent drainage continued for over a year. In none of the cases did bronchial fistulae develop. The persistent infection was accompanied by coughing and, at first, by loss of weight and anorexia. Cultures taken from time to time showed many types of bacteria, with a predominance of hemolytic streptococci. X-ray examinations showed a shadow corresponding to the extent of the disease, which was about the size of the gauze ring. At autopsy heavily infected cavities were found surrounding the gauze ring and the gauze placed in the center of the ring. The diameters of the cavities were from 3 to 4 cm. The parietal and visceral pleurae forming their walls were from 2 to 5 mm. in thickness. The parenchyma of the underlying lung was not involved in the infectious process, nor were there any evidences elsewhere of the extension of the disease.

I wish to thank Dr. William M. Millar and Dr. Julian Chisholm for their assistance in the performance of these experiments.

Summary. The criteria for the existence of chronic empyema in dogs should be (1) that an infection, localized in the pleural cavity, should discharge pus for at least one year, which represents about one-seventh of the life cycle of the average dog; (2) that the infection should not heal spontaneously; and (3) that the infection should be associated with systemic symptoms. These conditions have been fulfilled by following the procedure outlined in the text.

5161

Histological Study of Skin Reactions in Anaphylactic and Tuberculin Type of Hypersensitiveness.

L. DIENES.

From the von Ruck Research Laboratory for Tuberculosis, Asheville, N. C.

It has been stated that the local inflammatory reaction in hypersensitive animals, at the site of antigen injection, has no morphological characteristics and especially that there are no morphological differences between the local reaction due to anaphylaxis and to tuberculin sensitiveness.[1] We noticed, however, that slight reactions, especially during the first hours after the injection of antigen, show marked morphological differences in the 2 types of hypersensitiveness. It is reasonable to expect that under the conditions men-

[1] Opie, E. L., *J. Immunol.*, 1929, **17**, 329.

tioned the differences are more pronounced than in strong reactions or in later stages of the reactions when the consequences of tissue injury and reparatory processes obscure the original reaction.

We studied the microscopic structure of skin reactions in more than 40 guinea pigs and examined about 180 skin reactions. We compared the different types of skin reactions in the same animal whenever possible. Reactions were examined, 1, 2, 6, 24, and 48 hours after the injection, and different antigens were used, as egg white, crystalline egg albumin, tubercle bacilli and a tuberculin preparation, obtained from the synthetic culture medium (Long) after concentration with ammonium sulphate and elimination of the substances precipitable by acids. For the study of the anaphylactic type of skin sensitiveness we used actively and passively sensitized guinea pigs, both normal and tuberculous. It was necessary to examine tuberculous guinea pigs, as the infection itself exerts under certain conditions an influence on the cellular reaction in the inflammatory processes. We examined tuberculin type of reactions, both with tuberculin and egg white.

The results of these investigations were uniform. Our observations concerning the skin reactions of anaphylactic type, which appears as a quickly developing and transient wheal, correspond to the detailed description given by Gerlach.[2] The development of edema and of the vascular reaction is followed soon by a more or less intense accumulation of leucocytes which in the stronger reactions is well developed one to 2 hours after injection and often is very intense. A slight, in tuberculous animals somewhat more intense, accumulation of mononuclear cells usually follows after the subsidence of the macroscopic reaction. In guinea pigs, we observe necrosis in anaphylactic type of reactions only rarely, even in the cases when a central hemorrhage develops.

In the tuberculin reaction, the sequence in which the different macroscopic and microscopic manifestations of the reaction develop, differs considerably from the above given description. In the slight reactions, often before any macroscopical sign of the reactions is apparent (2 to 6 hours after the injection) we find a strong accumulation of mononuclear cells around the blood vessels. This is the first symptom of the developing reaction, which precedes the symptoms from the side of the circulatory system. After 24 hours, even in very slight reactions consisting of a trace of redness and swelling, we find a strong infiltration with mononuclear cells in the whole area of reaction. Besides these cells in the central area of the reaction we

[2] Gerlach, W., *Virchow's Arch. Path. Anat.*, 1923, **247**, 294.

find more or less polymorphonuclear leucocytes. In the slight reactions, their numbr is small. In the stronger reactions the infiltration with polymorphonuclear leucocytes is intense but, in marked contrast to the reactions of anaphylactic type, it is associated with a strong infiltration with mononuclear cell forms. In reactions where a central necrosis develops, the early stages of the reaction correspond to the above given description. Later, the necrotic area is surrounded by a strong leucocytic infiltration, consequent to the tissue injury. If we produce a skin reaction with tubercle bacilli in the first few hours a strong infiltration with polymorphonuclear leucocytes develops around the bacteria. Adjoining this area we find the infiltration with mononuclear cells characteristic to the tuberculin reaction.

Tuberculous guinea pigs react sometimes to non-specific local irritations by accumulation of mononuclear cells. We observed slight but pronounced reactions of this type after injection of normal saline in the skin. This influence of the infection on the cellular response does not obscure the characteristic differences between the anaphylactic and tuberculin type of reactions, except in very slight reactions of anaphylactic type in which the cellular reaction (consisting of polymorphonuclear leucocytes) is very slight, both in tuberculous and normal guinea pigs.

The anaphylactic type of reaction is characterized besides the symptoms from the side of the circulatory system, which first develop, by accumulation of polymorphonuclear leucocytes. Mononuclear cells take part only later and in slight amount in the reaction. In the tuberculin reaction, the first and, in slight reactions, the predominant symptom is a strong infiltration with mononuclear cells. The symptoms from the side of the circulatory system and the infiltration with leucocytes follow in time the former mentioned reaction, and in the strong reactions it is apparent that they are consecutive to tissue injury. The differences between the 2 types of reactions are certainly not only of quantitative nature, but indicate differences in the intimate mechanism by which the symptoms of the reactions are produced.

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The First Manifestation of the Developing Hypersensitiveness.

L. DIENES.

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The response of tuberculous guinea pigs to the treatment with egg white, if introduced into tuberculous lesions, differs in 2 important points from the response of normal animals.[1] 1. In tuberculous animals all manifestations of the immunization process (the sensitization and antibody production) develop in a largely increased measure. 2. There develops often a strong tuberculin type of skin sensitiveness to egg white, which we never observe in normal animals. This usually precedes the other manifestations of the sensitiveness and for a few days it might persist in pure form. A little later it is followed by the development of acute anaphylaxis, the anaphylactic type of skin sensitiveness, the protracted anaphylactic shock and the appearance of antibodies in the serum.

These characteristics of the immunity response of tuberculous animals suggested the possibility that the immunization process of normal animals also passes through a phase corresponding to the tuberculin sensitiveness, which is only slightly developed and has so far escaped attention. The tuberculin type of skin sensitiveness is possibly the strong development of this early stage of the immunization process. Observations support this supposition, namely, that the slight skin reactions in normal guinea pigs soon (5 to 7 days) after treatment are in many respects different from the usual anaphylactic type of skin reactions and more like very slight tuberculin reactions. They often appear delayed and although very slight, persist for 48 hours, in marked contrast to the quickly developing and transient reactions in passively sensitized guinea pigs. The recognition of marked differences in the microscopical structure between the tuberculin and anaphylactic type of skin reactions, described in the foregoing note, offered us a method of determining to which type the slight reactions belong, and of testing the place of the tuberculin sensitiveness in the whole immunization process.

We performed the following experiment. Two groups of guinea pigs (each group 4 animals) were injected intraperitoneally with 0.4 and 2 mg. egg white respectively. On the 5th, 6th, 7th and 8th day after treatment we cut out the skin tests produced with 0.2 mg.

[1] Dienes, L., and Schoenheit, E. W., *Am. Rev. Tuberc.*, 1929, **20**, 92; *J. Immunol.*, 1927, **14**, 9; 1928, **15**, 141, 153.

egg white, from one guinea pig in each group, 6 and 24 hours after the test injection. As controls we examined the skin tests of 3 non-treated guinea pigs. The skin reactions were very slight, consisting in a trace of redness and of swelling which after 24 hours was more pronounced than after 3 to 6 hours. The controls gave no reactions.

The results obtained were uniform; in the microscopical structure, the skin reactions were similar to the tuberculin reaction. A more or less intense infiltration with mononuclear cells occurred, which 6 hours after the injection was in several cases very pronounced around the blood vessels and was considerably increased in intensity in the 24 hour test. Leucocytes appeared only in moderate number. In the anaphylactic type of skin reactions in passively sensitized animals or after a longer period following the treatment, we find mainly an infiltration with polymorphonuclear leucocytes.

The experiments substantiated our supposition that in the immunization process the appearance of antibodies in the serum and the development of the usual type of hypersensitiveness is preceded by a stage similar in many respects to the tuberculin type of hypersensitiveness. The specific sensitiveness of the tissues in this first stage manifests itself in the accumulation of mononuclear cells at the site of injection with a relatively slight influence on the circulatory system. We do not want to express, at present, an opinion concerning the origin and nature of the cells taking part in the cellular reaction. It is very probable that the tuberculin type of skin sensitiveness is an exaggerated development of this first phase of the immunization process, under the increasing influence of the infection and it is hardly necessary to mention that the study of this first phase of the immunization process, which has so far escaped attention presents great interest in connection with the development of immunity in infectious diseases.

5163

An Experimentally Produced Bigeminal Arhythmia in Rabbits.*

WILLIAM F. ALLEN.

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Confirmation is made of Kratschmer's[1] and Koblanck and Roeder's[2] observations that a premature systolic arhythmia occurs in tracheotomized rabbits after insufflation of irritating vapors and from mechanical and faradic stimulations of the nasal septum before and after double vagotomy. It also follows faradic stimulation of the central end the vagus and insufflation of benzol into the trachea. In one instance it came on while the small intestine was being uncoiled. It does not follow faradic stimulation of the central end of the lingual, phrenic, depressor, ulnar and sciatic nerves or the peripheral end of the vagus and cervical sympathetic trunks.

Simultaneous apical beat, carotid pressure and vena cava tracings show the right atrium to be beating normally while the left ventricle has a bigeminal arhythmia and electrocardiograms demonstrate this arhythmia to be of left ventricular origin.

It would appear that this arhythmia is not due to a direct peripheral nerve reflex from 2 observations: (a) that it never comes on immediately with the insufflation stimulation as is the case of the arrested respiration and the rise in blood pressure; (b) that it requires a very powerful stimulation or sometimes a number of stimulations to produce it.

In view of the fact that this bigeminal arhythmia normally follows an arrested respiration, a rise in blood pressure and a slowed and strength-pulse it is necessary to show that these secondary factors and certain resulting internal secretions are not the actual cause of the arhythmia.

That an arrest of respiration did not elicit this arhythmia from asphyxia or lack of stimulations from failure to breathe is shown by experiments where no bigeminal pulse results from occlusion of the trachea or from inhalation of air highly concentrated with carbon dioxide. On the other hand this 'insufflation' arhythmia is readily obtained in rabbits having their thoraces opened on both sides and

* This paper was presented before the Section on Experimental Biology and Medicine at the Pacific Coast Branch of the Amer. Assoc. for Adv. of Sci., Eugene, June 19, 1930.

[1] Kratschmer, *Kreislauf. Sitzungsb. d. Wiener Akad.*, 1870, **62**, 147.

[2] Koblanck und Roeder, *Pflüger's Arch.*, 1908, **125**, 377.

breathing by artificial respiration. Apnea following forced artificial respiration has not resulted in a bigeminal pulse.

Numerous very similar and more pronounced rises in blood pressure than those which follow insufflations of benzol have been produced from occlusion of the abdominal aorta and from intravenous injection of adrenalin without evoking an arhythmia. On the other hand this arhythmia has been obtained from benzol insufflation when blood pressure has been prevented from rising by the use of an equalizer, the intravenous injection of ergotamine and from sectioning the spinal cord in the middle thoracic region. Many bigeminal pulse records have been obtained from insufflations without being preceded by any pulse changes; while the enormous slowing and strengthening of the pulse following occlusion of the trachea has never resulted in a bigeminal arhythmia. It is obvious also that the normal changes in blood pressure during insufflations are not contributory to anemia of the brain.

All humoral factors such as increased secretions from the adrenals, thyroid, liver, etc., are excluded as factors for producing this arhythmia by several cross circulation experiments, where the inner juxtaposed carotids of 2 rabbits were joined. An arhythmia produced in one animal from insufflation was never carried over to the other by means of the circulation.

That the carotid sinus of Herring is not the peripheral center for producing this arhythmia is shown by the absence of a bigeminal pulse in all of the mechanically produced rises in blood pressure and by a large number of 'insufflation' arhythmias obtained from the femoral arteries after both carotid sinuses had been removed.

That an impulse or series of impulses capable in some manner of producing a bigeminal pulse (probably arising somewhere in the *formatio reticularis* of the brain stem) descends the spinal cord to the left ventricle by way of the sympathetics is demonstrated by the following tests: (1) Transecting the spinal cord at the level of the 6 C vertebra always blocked an arhythmia from benzol insufflation; while sectioning the spinal cord from the 3-6 T vertebrae sometimes permitted an 'insufflation' arhythmia and sectioning the spinal cord at the 7-9 T always permitted the 'insufflation' arhythmia. (2) Removal of both stellate ganglia always blocked the 'insufflation' arhythmia, while removal of one stellate and sectioning the opposite sympathetic cord below the stellate generally permitted the 'insufflation' arhythmia. (3) Transecting the spinal roots at the level of the 7 C, 1 and 2 T also blocked the 'insufflation' arhythmia.

Over one hundred animals were used in the various phases of this problem.

5164

Electrodialysis in Application to Some Biological Studies.

WILLIAM DEVRIENT, STEPHEN THYSSEN AND BORIS SOKOLOFF.
(Introduced by Leo Loeb.)

From the Department of Pathology, Washington University School of Medicine, St. Louis, Mo.

Among the first to use electrodialysis in biological studies were Bronfenbrenner,[1] who constructed a special apparatus for the isolation of bacteriophage, and Murphy,[2] who used this apparatus in cancer research. One of us[3] has already reported about the difficulties which might possibly arise in using this apparatus for biological investigations. For this reason an improved apparatus was constructed for our research by Dr. N. Michailowsky. The apparatus differs from the older model in the construction of the cell in which the electrochemical process is carried out. This cell is divided into 3 parts, namely: anode, cathode and middle chamber. Between the 2 electrode chambers and the middle chamber 2 Norton Alundum Membranes can be introduced. These diaphragms, which have a diameter of 3½ inches may be combined with special Ultra or Cellafilters. The electrodes are exchangeable and, therefore, different metals can be used as electrode materials. Means for proper agitation and for temperature readings are provided. It is possible to use all 3 chambers for experiments and to study the electrochemical effects of anode, cathode and middle chamber separately. This apparatus can be operated by direct or alternating currents.

In order to study the behavior of living tissue in the described apparatus, we began investigations on *Paramaecium caudatum,* chicken-sarcoma Rous and other cancerous tissues as Flexner carcinoma and sarcoma No. 10 and No. 39.

The experiments on paramaecium were carried out in the middle division; there was at the same time a continuous circulation of water in the other 2 divisions.

We found that the voltage as compared with amperage has a relatively slight influence on the mortality and therefore we maintained a constant potential of about 100 volts in our experiments. A current of 0.005 Amp. and 100 volts which is equal to 0.5 watt can be withstood practically indefinitely without any harmful effects, but a stronger current seems to be fatal.

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[1] Bronfenbrenner, J., *Gen. Physiol.*, 1926-27, **10**, 23.

[2] Murphy, J., *Rev. of Int. Conf. of Cancer,* London, 1928.

[3] Sokoloff, B., *C. R. Soc. Biol.*, Paris, 1929, **150**.

We extended the study on paramaecium and made a few preliminary experiments observing the effects of electromagnetism at a temperature of 35° C. A magnetic field with a strength of more than 5000 gauss applied for several hours had a paralyzing effect on these organisms. After removal from the magnetic field they regained their former activity.

A similar study to that on paramaecium has been carried out on the above mentioned tissues. The exact survival time was determined by many series of grafts made in chickens with the sarcoma Rous and in rats with other tumors mentioned, after they had been exposed to electrolysis. All three chambers of the apparatus were used for the experiments on cancerous tissues. It is interesting to note that sarcoma Rous is about 10 times more resistant to the electric current than the other 3 cancerous tissues. The anode and middle divisions kill chicken sarcoma, while the cathode chamber does not show an appreciable effect. On the other hand paramaecium and the tumors are killed in the cathode chamber as well as in the other 2 divisions. It is furthermore of interest that similar relations exist between the mortality curves of sarcoma Rous and paramaecium. During the above described experiments the pH of the applied substances was not changed appreciably by the electric current.

5165

Electrochemical Behavior and Electromigration of Adrenalin.

WILLIAM DEVRIENT, STEPHEN THYSSEN AND BORIS SOKOLOFF.

(Introduced by Leo Loeb.)

From the Department of Pathology, Washington University School of Medicine, St. Louis.

We made use of the apparatus described in the preceding paper to study the electrochemical behavior of adrenalin.

If adrenalin in aqueous solution is added to the middle division of our apparatus, a current of 1 Milliamp. and 4 volts will effect a migration of this substance in the direction of the electrodes. It will be noted that adrenalin passes through the Norton Alundum discs used as diaphragms as well as through various Zsigmondy filters. All qualitative determinations of adrenalin were made by means of iron chloride, gold chloride and Folin's reagent. Quantitative experiments showed that the migration of adrenalin in the direction

of both poles occurs unequally. After the addition of alkali or alkaline salts to a solution of adrenalin hydrochloride a greater amount of adrenalin migrates in the direction towards the positive pole, while the addition of acid or acid salts causes a more marked migration in the opposite direction. The quantitative determination was carried out by the method of Folin as modified by Hitchcock and Benedict, using the colorimeter of Dubosque.[1, 2, 3]

We also made investigations concerning the electrolysis of adrenalin. We investigated first the influence of various electrode materials on the rate of decomposition of adrenalin. Carbon electrodes seemed to be the least efficient electrode material, while the metals Ni, Pt, Ag, Cu, Fe, Hg, represent a series with increasing efficiency. The rate of decomposition of adrenalin by electrolysis in the 3 divisions of the apparatus is shown in the following table, which may serve as an example of many other similar cases. The experiments were carried out at a temperature of 25° C. using Pt electrodes.

TABLE I.

Time in minutes	Volts	Amp./ squ. dm.	Anode Div. mgr/min.	pH	Middle Div. mgr/min.	pH	Cathode Div. mgr/min.	pH
15	7.0	0.9	0.05	7.0	0.07	7.5	0.13	7.5
30	7.0	0.9	0.02	4.5	0.09	9.3	0.15	9.5
45	9.0	0.8	0.015	4.2	1.00	10.0	0.24	10.0
60	6.0	0.8	0.07	4.0	1.30	10.0	0.35	10.0

J. Roest[4] had previously determined the rate of oxidation of adrenalin in alkaline solution (pH 7 to 7.2) to be about 0.3 to 0.5 ccm. per minute, but it could be as high as 3.0 ccm. per minute (pH 7.4). It can be seen from our table that the rate of decomposition in all 3 divisions is less than that found by Roest. Therefore, it can be stated that an electric current if not of excessive strength will within certain limits not attack the cathechol group of adrenalin.

If minute quantities of iron chloride are added, the electrolytical decomposition of adrenalin will be considerably accelerated.

In the anode division decomposition products like diketo-quinones, quinhydrone, aldehydes and other ketones were found, while in the cathode division hydroquinone, p-cresole and cyclic alcohols were present in addition to other substances which we have not yet determined.

[1] Folin, O., and Macallum, A. B., Jr., *J. Biol. Chem*, 1912, **13**, 363.

[2] Folin, O., and Denis, W., *J. Biol. Chem.*, 1913, **14**, 95.

[3] Benedict, S. R., and Hitchcock, E. H., *J. Biol. Chem.*, 1915, **20**, 619.

[4] Roest, J., *Biochem. Z.*, 1926, **176**.

5166

Changing a Right-thread Helix into a Left or Vice-versa; with Demonstration.

JOHN AUER.

From the Department of Pharmacology of St. Louis University School of Medicine.

In an earlier note[1] it was stated that free sections of fibrin filament were observed moving slowly forward and backward over the face of a red corpuscle without turning around; that fibrin filaments exhibited occasionally a faint diagonal striation indicating a left or right screw-thread; that the same filament showed at one time apparently a left thread and at another a right thread or vice versa; and also that a microcyte touching a red corpuscle at the point of attachment of an actively motile fibrin filament or flagellum, moved slowly first in one direction and then in the opposite direction. This reversal of direction in movement can be explained readily by utilizing the observation that a left helicoid apparently changed into a right helicoid, for with a given rotational direction, either right or left, the helicoid moves in one direction in a resistant medium when it possesses a left twist and in the opposite direction when the twist is right-handed.

That a right helix can change into a left or vice versa may be demonstrated easily in a model. If about 6 turns are cut from a small spool of spring-brass wire (B & S 19) a helix, generally right twist, will be secured. If the ends of this helix are firmly held in pin-vises, the coils moderately stretched apart and the helix rotated so that one end rotates slightly faster than the other, then a change of screw-thread will occur if the rotation is such that the coils widen and decrease in number. For example, in a wire helix with a right-hand spiral and rotating to the right (clockwise) under the conditions mentioned above, the change of twist generally shows itself first in one or the other terminal loop. This terminal loop suddenly exhibits a *left* twist and is connected to the rest of the helix which is still right twist, by a curved U shaped section (Node of reversal). On continuing the original rotation the node of reversal travels away from its site of formation and the number of loops with a left twist increases until the entire helix has changed its spirality. Now the originally right-handed helix rotating to the right has been changed to a *left*-handed spiral rotating to the right

[1] Auer, J., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 618.

(clockwise). It is clear that such a process must bring about a 180° change in the direction of travel.

If, on the other hand, the rotation of the helix is such that the number of loops increases and their diameter decreases, then sooner or later a complicated spherical skein or series of skeins is formed. This latter process is apparently active in the production of beaded fibrin filaments described and figured previously.[2]

This mechanism of changing a right helix into a left or vice versa has never been described to my knowledge. The whole process may be followed with comparative ease in a wire model and will be described and figured more fully in a final paper.

Helicoid structures are exceedingly common in nature, animate as well as inanimate; they may be microscopically small as in unicellular organisms or the tissues of plants and animals[3]; readily perceptible to the unaided eye as in tree-trunks, and climbing plants or tendrils (See Fig. 1); or they may be infinitely large, as in the whirling spiral nebulae of stellar space.

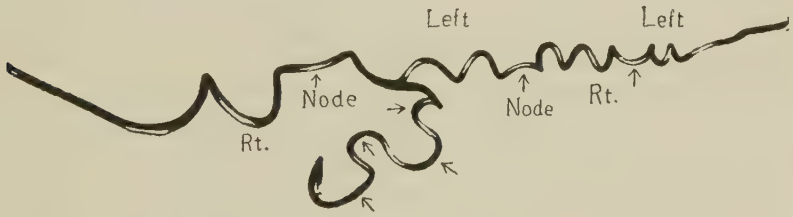

FIG. 1.

Grapevine tendril. Nodes of reversal are marked with arrows. L = left spiral. R = right spiral. Note change of twist after each node of reversal; also the series of 4 consecutive nodes.

If one assumes that all motion, molar, molecular and sub-molecular is helicoid, a number of processes become more accessible to our understanding. For example, the deviation of polarized light by optically active substances; the change of levo-compounds into dextro or racemic forms; the striking difference in physiological activity exhibited by levo and dextro forms, all appear to be explicable on this basis. In the final paper these aspects will be developed more fully.

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[2] Auer, J., loc. cit., 619.

[3] Auer, J., PROC. SOC. EXP. BIOL. AND MED., 1926, **23**, 379. See figure illustrating the article.

5167

Growth-Promoting Power for Planarian Worms of Eosinophilic and Basophilic Cell Groups in Anterior Pituitary.

ROSALIND WULZEN AND ALICE M. BAHRS.

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It has been demonstrated by Smith and Smith[1] that the injection of pituitary substances into hypophysectomized tadpoles produces 2 different effects according to the region of the anterior pituitary used. If the darker central core composed of basophil cells and chromophobes is injected the tadpoles grow more slowly and undergo metamorphosis more rapidly, while if one injects the surrounding lighter tissue, composed of eosinophil cells and chromophobes, the tadpoles grow unusually large. Although the separation of eosinophil and basophil cells in the pituitary is not complete, there is a sufficient predominance of the one in the outer portion and of the other in the central core to indicate that the growth stimulating principle arises from the eosinophil cells while that reacting with the endocrine system arises from the basophil cells.

Evans and Simpson[2] have also presented experimental evidence that 2 hormones are secreted by the anterior pituitary, one being a growth-promoting principle while the other reacts with the endocrine system. They consider their experiments to be concordant with the view that the one hormone is produced by eosinophil cells while the other is produced by basophil cells.

It seemed worth while to apply another test to determine whether the portion of the pituitary containing the greater number of eosinophil cells possesses a growth-promoting power exceeding that of the portion which contains the greater number of basophil cells. We used groups of planarian worms *(Planaria agilis)* newly regenerated from tails cut from our stock. Each group consisted of 30 worms, 6, 7, and 8 mm. long, and had a total length of 225 mm. The worms were fed twice a week over a period of 4 weeks when the total length of each group was again ascertained. The first group was fed liver from freshly killed rabbit, the second was fed strips from the outer, light-colored portion of anterior pituitary of freshly killed beef, while the third group received the reddish inner core of several beef, anterior pituitaries. All groups ate their food with great readiness.

At the conclusion of the experiment it was found that the worms

[1] Smith, P. E., and Smith, I. P., *Anat. Rec.*, 1923, **25**, 150.

[2] Evans, H. M., and Simpson, M. E., *J. Am. Med. Assn.*, 1928, **91**, 1337.

fed upon liver had increased in length 188 mm. or 83.5%, those fed upon the outer, acidophilic portion of anterior pituitary had increased 125 mm. or 55.5%, while those fed upon the inner, basophilic portion had increased in length 24 mm. or 10.6%. Thus the predominatingly acidophilic portion of the pituitary had manifested a growth-promoting power 44.9% greater than that of the predominatingly basophilic portion. Stating these results in terms of averages, they are as follows: At the beginning of the experiment both groups showed means of $7.50 \pm .07$ with standard deviation .548. At the conclusion of the experiment the group fed the basophilic portion showed a mean of $8.31 \pm .11$ and standard deviation .906, while the group fed the acidophilic portion showed a mean of $11.66 \pm .15$ and a standard deviation of 1.392.

It will be observed, however, that the growth-promoting power of liver exceeded the best pituitary growth by 28%. Whether there is inherently greater power in the liver or whether some factor of antagonism is at work in the pituitary tissue we have yet to determine. The work of Evans and Simpson[2] indicates an antagonism between the 2 hormones of the anterior pituitary, that promoting growth and that affecting the endocrine system.

Our investigation indicates that the predominatingly acidophilic portion of the anterior pituitary possesses a greater growth-promoting power for planarian worms than the predominatingly basophilic portion This result was obtained by feeding the fresh gland to normal worms.

5168

Further Observations on Experimental Aortic Insufficiency. III. Factors Accountable for the Systolic Collapse of the Central Pressure Pulse.

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The arterial pressure pulse during aortic insufficiency is described as having both a "water hammer" and "collapsing" characteristic, the former term referring to the impression given by the abrupt and great systolic rise, the latter, to the tactile and graphic effects of the steep gradient of its decline. Stewart[1] first directed attention to

[1] Stewart, H. A., *Arch. Int. Med.*, 1908, **1**, 102.

the fact that the chief decline of pressure precedes the dicrotic wave in the peripheral pulse and from this drew the conclusion that it must be systolic in time rather than diastolic, as is commonly taught. Subsequent improvements in methods of graphic registration, together with a clearer understanding of the physical changes involved in pulse transmission, have shown that this inference was not justifiable. The writer[2] found that the diastolic gradient of the central arterial pulse is predominately affected and chiefly concerned in the collapse and lower diastolic pressure. The analyses of Frank[3] have shown that the end of systole does not correspond to the rise of the dicrotic wave of the peripheral pulse but coincides with an uncertain and not easily determinable point on that part of the descending slope which precedes the dicrotic wave. Nevertheless, careful inspection of optical records obtained from experimental and clinical valvular insufficiency reveals the fact that the decline of pressure previous to the apex of the V-shaped incisura is also greater (*Cf.* Figs. 1-2). Though not strictly correct, we may refer to this as the "systolic collapse".

The cause of this phenomenon has not been analyzed. Obviously it can be due to either or both of 2 possible alterations: the systolic pressure maximum may be higher or the incisural apex may occupy a lower position on the pressure curve. A determination of the fac-

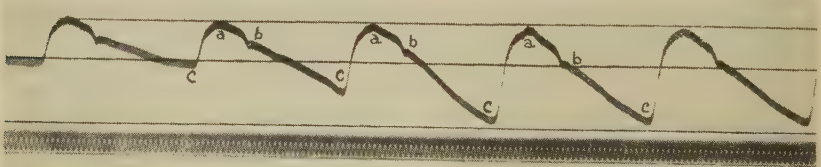

FIGURE 1

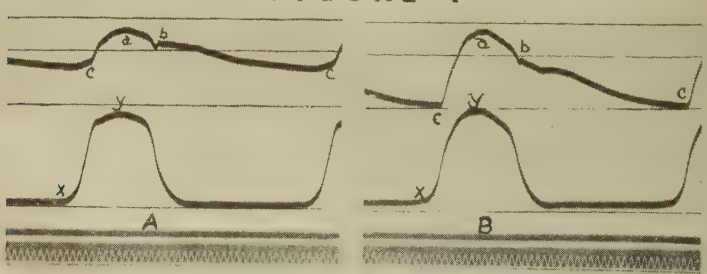

FIGURE 2

[2] Wiggers, C. J., *Arch. Int. Med.*, 1911, **8**, 17.

[3] Frank, O., *Sitzungsberichte der Gesellsch. f. Morphol. u. Physiol. in Munchen*, 1926, **37**, 33.

tor actually concerned is of importance in completing our conceptions of the dynamics of aortic insufficiency; for if the greater systolic pressure drop be due solely to the former it would be adequately accounted for by the increased force of ventricular contraction, but if it be due to an absolute decrease in the position of the incisura a late systolic or very early diastolic loss of pressure must be concerned.

In order to study the question experimentally, intra-aortic and intra-ventricular pressure pulses were recorded optically by means of the author's manometers, and temporary valvular insufficiency was produced by a method previously described.[2] Sixty-four experiments were made on 5 different dogs, under morphine and barbital anesthesia.

Typical records are reproduced in Figures 1 and 2. A glance at the former establishes the fact that the abrupt post-incisural decline of pressure (b-c) is predominately responsible for the lower diastolic pressure (c) and the larger pulse pressure (c-a). However, the pre-incisural drop (a-b) is also typically greater, due to a lower position of the incisural apex (b). Figure 2 comprises 2 segments of records from another animal. Segment A serves as a normal control, segment B represents the pressure changes shortly after production of an aortic insufficiency. In addition to the features exhibited in Figure 1, this curve shows that the greater pre-incisural drop is due in part to a lower position of the incisural apex (b) and in part to a higher pressure maximum (a). A study of intra-ventricular pressure curves (lower records) shows that this is due to a more vigorous contraction, the initial (x) and the ventricular pressure maximum (y) both being elevated.

An analysis of 64 sets of similar records showed that, as in Figure 1, the greater systolic collapse was due exclusively to a lower position of the incisura in 35 instances. In some of these the systolic pressure maximum remained unaltered, in others it decreased slightly. In 29 experiments, the systolic pressure maximum was elevated in addition, as in Figure 2. In three instances only, the incisural position was unaffected and the greater systolic collapse was due entirely to a higher pressure maximum.

Conclusions. (1) The pronounced systolic fall of pressure which accompanies the steeper diastolic decline characteristic of aortic insufficiency is practically always associated with a lower position of the incisural apex. (2) Therefore, a late systolic or early diastolic loss of pressure from the arterial system must occur as a result of insufficient valves. (3) An elevation of the systolic

pressure maximum may or may not contribute to the greater systolic collapse, depending on the degree to which physiological compensation operates in a particular heart.

5169

A New Tri-atomic Alcohol from the Urine of Pregnant Women.

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From the Department of Biological Chemistry, St. Louis University School of Medicine.

A new alcohol differing from the pregnandiol of Marrian[1] and Butenandt[2] and also from the crystalline ovarian hormone isolated by Doisy,[3] Thayer and Veler, Butenandt,[4] Laquer[5] and Marrian[6] has been obtained from the urine of pregnant women.

This alcohol has been isolated in the form of snow white crystals. The melting point by the open beaker method of 5 different preparations was 273°, 273°, 273°, 272.3°, 272° (uncorrected). The crystals melted sharply without decomposition.

The molecular weight determination by Rast's micro procedure gave an average value of 294. The iodine numbers of 3 different preparations were 85.3, 86.2, and 88.5. The average of these values, 86.7, permits one to calculate a molecular weight of 292.8 if one double bond is assumed.

Determination of the number of hydroxyls in 2 samples by the procedure of Peterson and West[7] indicates that 3 atoms of oxygen exist in this form. Found 124, 129; theory 129 gms. of $CH_3C=$ 0 per mole. The average molecular weight of the triacetyl derivative is 410. M. P. 126° uncorrected.

The specific rotation of a 0.322% solution in 95% ethyl alcohol at 28° in a 2 dm. tube with a sodium flame was +68.3°. Another sample (0.148%) gave a value of +72.8°.

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[1] Marrian, G. F., *Biochem. J.*, 1929, **23**, 1090.

[2] Butenandt, A., *Ber. d. deut. chem. Ges.*, 1930, **63**, 659.

[3] Doisy, E. A., Thayer, S. A., Veler, C. D., *J. Biol. Chem.*, 1930, **86**, 499.

[4] Butenandt, A., *Naturwissenschaften*, 1929, **17**, 879.

[5] Dingemanse, E., de Jongh, S. E., Kober, S., and Laquer, E., *Deut. Med. Wochnschr.*, 1930, **56**, 301.

[6] Marrian, G. F., *Biochem. J.*, 1930, **24**, 435.

[7] Peterson, V. L., and West, E. S., *J. Biol. Chem.*, 1927, **74**, 379.

Qualitative analysis did not detect sulfur, halogens or nitrogen. Quantitative micro analysis: carbon 75.15%; hydrogen 8.22%. Calculated for $C_{18}H_{24}O_3$, carbon 74.95%; hydrogen 8.39%; mol. wt. 288.

Physiological Activity. Injections of minute quantities (recrystallized 10 times) of this new alcohol cause opening of the vagina of sexually immature rats and mice. Subcutaneous administration to spayed adult rats produces cornification as judged by the vaginal smears. Cornified cells appear usually on the fourth or fifth day following the injections counting the day of injection as the first. The presence of numerous cornified cells continues for several days. The response to this substance is quite different from the response to theelin. Theelin, like purified extracts of hog liquor folliculi, generally shows the peak of its action on the third day with a rapid return to the dioestrous type of smear.

5170

Physiological Reactions of Goldfish with Severed Spinal Cord.

DAVENPORT HOOKER.

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So far as the writer is aware, the work of Koppányi and Weiss,[1] and Pearcy and Koppányi[2] on goldfish, and that of Nicholas[3] on Fundulus embryos (none of which has been published in full), constitute all that has been done on spinal cord section in teleosts. The first two of these papers report functional regeneration of the severed cord. Nicholas definitely failed to secure any restoration of function or of anatomical continuity.

To further test the matter, a study of the results of spinal section in the goldfish *(Carassius auratus)* has been undertaken. To date, 25 small goldfish have been subjected to operation on the spinal cord. The animals were anesthetized with chloretone, transluminated in a dark room, and the spinal cord presumably cut with a knife in the region marked by the beginning of the dorsal fin. Subsequent histological examination has proved cord section in the majority of these.

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1 Koppányi and Weiss, *Anz. d. Akad. d. Wissen. Wien*, 1922, **59**, 206.

2 Pearcy and Koppányi, PROC. SOC. EXP. BIOL. AND MED., 1924, **22**, 17.

3 Nicholas, *Proc. Nat. Acad. Sci.*, **13**, 695, and personal communication.

Koppányi and his coworkers state that all goldfish in which the spinal cord has been severed lie on their sides. This was not found to be true in this investigation. Some remained erect, while others lay on their sides when at rest. All were capable of swimming voluntarily. As some specimens which lay on the side did not have sectioned cords, spinal section is not the cause of this behavior. The nature of the injury producing this posture is, as yet, unknown, though experiments to determine it have been carried out.

Light tactile stimulation on the body fails to produce any response in the normal, or in the spinal fish if applied cephalad to the lesion. Stronger stimulation causes swimming in normals and, if applied in front of the lesion, in spinal fish. In every case, spinal section releases a typical avoiding reaction to all tactile stimulation in the area behind the lesion. This avoiding reaction is found only in spinal fish, as proved by histological examination. It is not to be elicited in front of the lesion in spinal fish nor in normal fish.

The fins innervated from behind the lesion are not moved voluntarily, but the caudal part of the dorsal fin enters into the avoiding reaction. Progression in spinal fish is produced by the pectoral fins and the musculature of the body in front of the lesion. The tail is not used, though its fin is often passively spread by water currents created by the pectoral fins, simulating voluntary motion.

As yet, no fish with spinal lesion has survived more than 24 days. Though histological examination of sections of these fish has not afforded a complete series of stages, no evidence indicating regeneration of the cord has been observed. On the contrary, cellular lysis appears to occur in the goldfish as in the rat (Hooker and Nicholas[4]; Nicholas and Hooker[5]; Hooker and Nicholas[6]). If cytolysis should prove to be as general as now appears, cord regeneration in this form is probably impossible.

[4] Hooker and Nicholas, *Anat. Rec.*, 1927, **35**, 14.

[5] Nicholas and Hooker, *Anat. Rec.*, 1928, **38**, 24.

[6] Hooker and Nicholas, *J. Comp. Neur.*, 1930, **50**, 413.

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The Calcium Content of the Body in Relation to That of the Food.

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The importance of calcium in normal development accompanying growth, and the purely scientific interest in the so-called law of the minimum, have both emphasized the desirability of more extended investigations of the relation between the calcium intake and the calcium content of the growing body.

In the present investigation, chemical analyses have been made of the bodies of large numbers of rats which, starting from comparable heredity and nutritional backgrounds and with all factors other than food maintained strictly uniform, had received food of differing calcium content. The calcium content of the diet was varied in two ways: (1) by varying the ratio of two natural foods of widely different calcium content wheat and milk; (2) by feeding specially designed laboratory diets in which calcium, in the form of calcium lactate, was the sole variable factor. In both cases vitamin D was amply supplied.

It was thus found that individuals otherwise well nourished but with low calcium intake do grow up with calcium-poor bodies. It is also evident as a result of these experiments, that the body may be able to retain calcium at a sub-normal rate on a diet relatively poor in calcium and so at an abnormally late age may finally come to approximate a normal percentage of body calcium.

Application of these findings to the problem of normal human nutrition should prevent any relaxation of attention to adequate calcium supply in the food. Vitamin D as a "calcium mobilizing" factor is not a substitute for calcium. The body at birth has a low calcium content and its normal development involves a large increase not only in the *amount* but also in the *percentage* of calcium which it contains, and so requires a liberal supply of calcium in the food. Otherwise the growing body remains calcium-poor, even though it shows every external appearance of being well nourished.

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On the Instability of Brilliant Cresyl Blue.

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The employment of this dye (dimethyl diamino toluphenazine chloride) in biochemical work warrants the publication of this note on some of its properties.

The purified *dry* dye when extracted with *dry* ether, benzene or xylene gives no coloration to the solvent. Chloroform has the disadvantage that the dye salt is appreciably soluble in it.

Addition of water to the dye-solvent mixture results within 10 minutes in coloration of the non-aqueous phase. This coloration increases progressively with time, with increased temperature and with extremes of pH. It is not a result of interaction of dye with the non-aqueous solvent, for independent oxidation-reduction titrations in aqueous solutions show the presence and accumulation of secondary systems as contact of dye with water is prolonged. The conclusion must be, therefore, that *brilliant cresyl blue remains 'pure' only so long as it is kept out of contact with water.* This instability seems to be of the same kind and degree as that found in methylene blue.

The nature of the secondary products is unknown. It is suspected that they are demethylated and deaminated derivatives of the parent dye. At least 2 are present. One has the properties of an oxazone. It is a very weak base with dissociation constant less than 10^{-13} and its solutions from pH 1 up to normal KOH are colored red with a golden fluorescence. The second product has the properties of a stronger base, with a dissociation constant near 10^{-6}.* Its salt is colored blue and the base orange.

Brilliant cresyl blue itself is a stronger base with a dissociation constant of 10^{-3}.

* This corresponds to the value assigned by Irwin, *J. Gen. Physiol.*, 1925, **9**, 561, to the dissociation constant of brilliant cresyl blue.